` **Jimma University**

Institute of Health Sciences

Public Health Faculty

Department of Environmental Health Sciences & Technology

Ready-to-eat food contamination with antibiotic-resistant bacteria in the Debre Tabor University student cafeteria

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A thesis submitted to the Department of Environmental Health Sciences and Technology, Faculty Public Health, Institute of Health Sciences, Jimma University, in partial fulfillment of the requirements for Master of Science degree in Environmental Health

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Jimma, Ethiopia

APPROVAL SHEET

As thesis research advisors, we hereby approve that we have read and evaluated this thesis prepared under our guidance by Chalachew Yenew entitled "**Ready-to-eat food contamination with antibiotic-resistant bacteria in the Debre Tabor University student cafeteria".** we recommend that as fulfilling the thesis requirement.

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Co-advisor Signature Date

As a member of the board of examiners of the MSc thesis open defense examination, we certify that we have read and evaluated the thesis prepared by ChalachewYenew and examined the candidate. We recommend that the thesis be accepted as fulfilling the thesis requirement for the degree of Master of Science in Environmental Health.

_____ _____ _____

Chairperson Signature Date

_____ _____ _____

Internal examiner Signature Date

Declaration

I, the undersigned, declare that this is my original thesis work, has not been presented for a degree in this or another university, and that all sources of materials used for this have been acknowledged.

Chalachew Yenew Signature _____Date_____

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ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis Of Variance
ETB	Ethiopian Birr
SPSS	Statistical Package for Social Sciences
CIs	Confidence Intervals
FBDs	Food-Borne Diseases
AMR	Antimicrobial Resistance
WHO	World Health Organization
US	United State
EPA	Environmental Protection Agency
RTF	Ready-to-eat Food
TCBS	Thiosulphate Citrate Bile and Sucrose
E. coli	Escherichia coli
ARR	Antibiotic Resistance Rate
MIZ	Mean Inhibition Zone
MDR	Multidrug Resistance

ABSTRACT

Background: Foodborne diseases (FBDs) are a significant public health issue in both developed and developing countries often resulted in deteriorating clinical outcomes, high morbidity, and mortality. Currently, Both Societies face a global problem of emerging and re-emerging foodborne antibiotic-resistant infections and outbreaks.

Objective: This study aimed to determine the contamination of ready-to-eat food (RTF) with antibiotic-resistant bacteria and major associated factors in the Debre Tabor University student cafeteria, Northwest, Ethiopia,2020.

Methods: A laboratory-based cross-sectional study was conducted by following standard microbiological methods to isolate and identify foodborne bacteria from ready-to-eat food. The disc diffusion method was used to performing the resistance profiles of the foodborne bacteria that were identified from ready-to-eat food in March 2020. Thirty samples of ready-to-eat-food, food utensils swab, and hand swab of the food handlers were collected. Besides, work experience, drug use characteristics, and educational status of the food handler's data were collected by using observational checklist and interview questions. The descriptive statistics, correlation, and linear regressions were used to analyze the data.

Results: Ready-to-eat food of the Debre Tabor University student cafeteria were contaminated with multiple antibiotics-resistance (MAR) Escherichia coli 43% (95% CI: 41.2%, 46.9%), Salmonella 36.7% (95% CI: 33.2%, 38.7%), and Shigella 20% (95% CI: 19.2%, 26.9%) with the overall multidrug resistant (MDR) level of 94.43%, 85% and 89.58% and multiple antibiotics-resistance (MAR) index of 0.6, 0.6 and 0.8 respectively.

Conclusions and recommendations: Multiple antibiotic-resistant bacterial contamination of ready-to-eat food was associated with poor personnel hygiene of the food handlers, work experience of fewer than 5 years of the food handlers, inappropriate drug use characteristics of the food handlers, lack of food safety training of the food handlers, educational status of less than or equal to secondary education of the food handlers, and poor sanitary condition of food utensils.

Preventive measures like the provision of food safety training to food handlers and strict follow up for implementation of good hygienic practices might improve ready-to-eat food safety.

Keywords: Antibiotic-resistance bacteria, Ready-to-eat food, Debre Tabor University, Ethiopia

CHAPTER ONE: INTRODUCTION

1.1 Background

According to World Health Organization, Foodborne diseases (FBDs) are becoming a major health issue for all, because of adverse effects such as high economic crises, worsened health outcomes, and high mortality in high and low-income countries. However, human beings have the right to get the food they eat to be safe, wholesome, and comfortable for consumption (WHO, 2015a).

Food is a possible route of highly prevalent infection and can easily be infected with the potential pathogenic microbes from farm to fork at any stage (WHO, 2015b). So that a great deal of attention is paid to food hygiene in production, handling, distribution, and serving of all food types that prevent food poisoning and other foodborne infections by breaking the pathogens transmission route (James et al., 2010).

The pathogenic bacteria are entering into the foods from different sources with regards to their loads and categories and increase in both numbers and development because food is a favorable environment for bacterial growth (Dennis, 2019).

They cause cross-contamination of ready-to-eat foods with pathogenic bacteria that have been associated with many foodborne outbreaks and epidemics (Draeger et al., 2018). Therefore, the crisis of improper food handling in mass-catering food establishments such as cafeteria and restaurants can be much higher than that of the household. Because of a large number of people may be simultaneously exposed to contaminated food items (US Academic Press, 2014).

Proper sanitation and hygiene at every stage help reduce the pathogenic bacteria load to the normally expected level in food and used to contain and prevent FBDs and outbreaks (Bibek, 2005).

Recently, antimicrobial resistance pathogens are common worldwide problems and also the Ministry of Health of the Federal Democratic Republic of Ethiopia on different meetings mentioned that all populations are challenged by a serious problem of endemic, emerging, and reemerging antibiotic-resistant bacterial infections and outbreaks (CDC, 2019; FDRE MOH, 2017).

1.2 Statement of the problem

Globally, AMR pathogens posed the most significant public health and economic threats where, ten million humans at risk with 700, 000 deaths, and 100 trillion USD of economic loss per year. Hence, these figures are assumed to increase significantly if preventive measures could not be done (Viens, 2015).

Nowadays, bacteria are greatly resistant to almost all antibiotics that were before active against them. Amazingly, there is no new group of antibiotics that have been revealed ever since 1987. For all these reasons, AMR pathogens are considered a 'slowly emerging disaster' and global common health issue (Regea, 2018; Viens, 2015).

Recently, the phenomenon of AMR pathogens in the surrounding has been more and more reported worldwide due to the overuse of antibiotics, antibiotic recommendation with no susceptibility check, improper use of antibiotics, self- medication, poor AMR pathogens regulation, and policy (Jim, 2016; Katrin, 2018).

The existence of AMR pathogens in foods is becoming an ever more community health problem worldwide due to the overuse of antibiotics in animal feed, plant growth promotion, food additives and preservation, and human medication (Levy, 2011).

The prevalence of AMR foodborne pathogens all along the food chain and how they can arrive at consumers not well studied. However, it is reasonable to believe that AMR bacteria from the production can enter and stay in the food system and (re)contaminate, continue to exist, and/or develop in food or food environments resulting in their presence of both on raw and ready-to-eat products at the consumption stage (Taban, 2019; Verraes et al., 2013).

Much African AMR pathogens investigation and surveillance results show that most pathogens are 50 percent to 100 percent resistant to widely used antibiotics such as ampicillin and cotrimoxazole. They contribute to more common treatment failure, increased morbidity, and mortality, chronic infection, increased infant and child death, and other worsening conditions (Ampaire et al., 2016).

These major threats may occur due to lack of a comprehensive policy and plan to address antibiotic-resistant pathogenic microbes; weak antibiotics regulatory capacity, and circulation of substandard/counterfeit antibiotics; lack of antimicrobial surveillance strategies; weak laboratory capacity on AMR testing and reporting; lack of essential laboratory reagents and

consumables; and limited quality assurance, and control protocols (Founou et al., 2018; Ndihokubwayo et al., 2013).

Despite, the limited laboratory investigation and surveillance quality mostly isolate Vibro cholera, Salmonella, Escherichia coli, and Shigella and they result in enteric diseases and other major communicable infections in Africa (Smith, 2019).

The increasing occurrence of AMR pathogens and their threats was a concern of the high and low-income countries (Aastha et al., 2019). In Europe gram-negative antibiotic-resistant bacteria like Salmonella, Shigella, E.coli, and Vibro cholera widely prevalent among the migrating population, and they result in great public health threats (Nellums et al., 2018).

The widespread emergence of AMR pathogens has become one of the most serious challenges in Ethiopia. The major reasons were antibiotic- drug misuse, drug prescription without susceptibility test, self-medication, and a long stay in the hospital environment (Tamiru et al., 2017).

Some experimental investigation and surveillance in Ethiopia show that high resistance levels of E. coli, Shigella, Vibro cholera, and Salmonella species have been reported resistant with frequently recommended antibiotics such as amoxicillin, penicillin, doxycycline, and cotrimoxazole (for instance, Salmonella species resistant to multiple antibiotics 100%) (Moges et al., 2014).

Mostly raw and ready-to-eat foods are the potential sources of the above AMR pathogens. As a result, many AMR foodborne infections and outbreaks have occurred (Wolde et al., 2016). Their occurrence is mainly due to prevailing poor food handling and sanitation practices, inadequate food safety laws, weak regulatory systems, unclean equipment and utensils, and lack of education for food-handlers, overuse of antibiotics, misuse of antibiotics, and also the prevalence rate and their major factors are rarely investigated in detail and under-reported (Founou et al., 2016; Hussen et al., 2019).

The food is a primary source and dissemination routs of AMR have been broadly mentioned in the academic literature. However, a compressive determination of the contamination level, identification of their major sources of contamination, and multiple antibiotics-resistance tests of AMR foodborne bacteria on ready-to-eat food of the University cafeteria were not well understood. Therefore, this study aimed to determine the ready-to-eat food contamination with AMR bacteria and associated factors in the DTU Student Cafeteria.

1.3 Significance of the study

According to the World Health Organization (WHO, 2011), there is a growing demand for food safety information at the international, national, and local levels. Yet, ready-to-eat food contamination with AMR bacteria and their associated factors not studied in Ethiopia. So that undertaking the study will help to fill the gaps with this regard.

Therefore, this study aimed to determine ready-to-eat food contamination with AMR bacteria and associated factors.

Moreover, this study will help the community, the government, and NGOs to develop an action plan and implement an appropriate intervention for AMR bacteria.

Furthermore, this study will also be used as a baseline for stakeholders who are interested to intervene in this area. In the same way, function as a baseline for researchers who wish to take on similar studies.

CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of AMR

Antimicrobial- resistance (AMR) is a broad term that refers to the capability of a microorganism to defend against the effects of medication that could previously treat them. while, antibiotic-resistant is a sub-part of an AMR, which is used only to bacteria becoming resistant to antibiotics (O'Neill, 2014; WHO, 2014).

AMR pathogenic infections are not easy to treat, require other medications, the combination of medications, or a high dose of antimicrobials. These approaches may be more costly, and highly poisonous or both. This is a conventional approach to fight antimicrobial -resistance. Microbes that are resistant to numerous antimicrobials are called multidrug-resistant (MDR). Those considered comprehensively drug-resistant (XDR) or entirely drug-resistant (TDR) are sometimes called "superbugs" (Adegoke et al., 2017; IACG, 2018).

Preventive measures include only the use of antibiotics as necessary, thus preventing the overuse of antibiotics and also addressing the main transmission routes of AMR pathogens (Johan &Malin, 2014; Kirienko et al., 2019).

Narrow-spectrum antibiotics are like better than broad-spectrum antibiotics when probable, as successfully and exactly targeting specific bacteria and less likely to cause resistance, as well as less toxicity (Jeffrey et al., 2017).

Health caregivers may reduce the spread of resistant infections by promoting the proper use of sanitation and hygiene for all groups of population (WHO, 2015a).

2.2 Transmission routs of AMR bacteria

AMR bacterial infections and outbreaks are the major concerns of public health in the past, the future, and today. Nevertheless, the conventional approach continues to be used to counter these infectious diseases and outbreaks that rely on new antibiotics, increase the dosage, and antibiotic combination as solutions. This led, increasing toxicity, and resistance. So, there is a larger requirement for alternative treatments and call for new antibiotic have been issued, Amazingly, new drug development is becoming rarer (Cassir et al., 2014; WHO, 2012).

On the other hand, the distribution of AMR genes and pathogens in the ecosystem is not well understood (Susanne et al., 2019). However, there is substantial scientific evidence that resistant

bacteria, resistant genes, and antibiotics will spread through the following paths (Johan and Larsson, 2015; UN-EPA, 2014).

2.2.1 Person to person

AMR pathogens can spread through direct human contact from one person to another person. Transmission may also occur indirectly, when someone coughs, for example. If a person contaminates surfaces with AMR pathogens of different utensils and equipment, these pathogens can be transferred to another person who touches the surface. To limit the spread of antibiotic-resistant bacteria and the risk of becoming a carrier of resistant bacteria, good hand hygiene is important (Emily et al., 2018).

2.2.2 Animals to humans and vice versa

Antibiotic-resistant bacteria can spread from animals to person, but also the other way around; from person to animal. animal pathogenic bacteria become resistant to first-line antibiotics, diseases become more difficult to treat, just as in humans (Pietro, 2016; Pirolo et al., 2019).

2.2.3 Food

Fruits, vegetables and other food products can become contaminated with feces and urine (contain 80% antibiotics, antibiotic-resistant bacteria, and antibiotic-resistant genes) and also directly contaminated by, waste materials (greater than 50% municipality solid waste contains unused and expired drug and wastewater treatment plant can not remove all antibiotics, antibiotic-resistant bacteria, and antibiotic-resistant genes), or it can be contaminated via the water used for cooking, drinking & washing (Stephanie, 2015; UN-EPA, 2014).

These may result in contamination of food contact surfaces, utensil contact surfaces, and also food handlers. Eating food contaminated with bacteria may directly cause an infection, such as diarrhea caused by Salmonella, Vibro cholera, Shigella, and E.coli. Resistant bacteria species may also be transferred to the normal gut flora of the consumer without causing an infection. The resistant bacterium can potentially cause infections later on and spread to other people. So, proper cooking and handling of food help to decrease the spread of infections as well as resistant bacteria (Börjesson et al., 2016; Davis et al., 2018).

2.2.4 Water

Bacteria can spread via drinking water or water supplies that are used for irrigation, washing utensils, and other types of equipment or other hygienic purposes. Antibiotic-resistant bacteria could have occurred in any water source such as drinking wells, rivers, and effluents from

wastewater treatment plants. For example, bacterial diseases including typhoid fever and cholera can spread via contaminated water (Nachiket et al., 2013; Steven et al., 2017).

2.2.5 Spread within health care facilities

Health facilities are hot spots for resistant bacteria because many sick people are in close contact with each other and antibiotic usage is high. And result in the introduction and spread of resistant-bacteria species. poor hygienic practices of an individual could help the high spread of resistant bacteria via the hands or clothes (Evelina et al., 2014).

2.2.6 Traveling

International travelers spread resistant bacteria across the world. On any day numerous million people will take a flight, and if a person carries a resistant bacterium. He/she will transmit them to another person. several studies have revealed that a huge proportion of international travelers acquire resistant bacteria during visits in areas where there is a high prevalence rate of resistant bacteria and their infections (Paul-Louis et al., 2013).

2.2.7 Trade

Meat, fruit, vegetable, seed, grain, and animal are imported and exported to or from different countries. This might be contaminated by antibiotic-resistant bacteria. Then the bacteria could be potentially spread all over the world (Grami et al., 2016).

Nowadays, the increasing prevalence of antibiotic-resistant bacteria on plant and animal food products are mainly caused by the overuse of antibiotics in humans (30%), animals (70%), 75% antibiotics present on aquaculture and also a high amount of antibiotic are absorbed by the plant (Stephanie, 2015; UN-EPA, 2014).

The spread of resistant-bacteria between the human, and animals and the growing of the occurrence of them in the environment are highly interconnected and complex (Henrik et al., 2015).

Poor hygiene, poor sanitation, and poor infection prevention and control are the three major interrelated contributing factors for the spread of resistant bacteria in health facilities, in the population as well as in food production (WHO, 2019).

2.3 AMR bacteria and food safety

In recent years there has been a dramatic increase in the prevalence of AMR bacteria in raw and processed foods. They have their ability to gain newly antibiotic-resistant distinctive character. Such newly emerging antibiotic-resistant bacteria were previously unknown to the food

industry. Since only a few studies were available on their existence in foods, but the epidemiological circumstances have changed with the advent and spread of them in foods due to the extensive use of antibiotics in food production; thus, antibiotic-resistant bacteria can reach the intestinal tract of humans (Muloi et al., 2018).

There is an acceptance that the transmission of many AMR bacteria primarily occurs from an infected person to other persons. highly resistant bacterial infections are no longer limited to hospital-acquired infections since foods are frequently contaminated with AMR bacteria and, hence, has started to become the possible source for the exposure of not only high-risk groups like vulnerable patients, but also whole public (WHO and FAO, 2019).

AMR bacteria are a major concern for the food industry as they could contribute to consumer trust loss and ultimately decrease in food demand as well as lead to more fatal diseases. From this point of view, the AMR bacteria in foods not only impose significant health threats but also causes significant but avoidable economic losses (Getie et al., 2019; Shea, 2012).

The sources of contamination must be well known to prevent and control the spread and occurrence of AMR infections and outbreaks through the food. There are several complex routes of transmission of these resistant bacteria along the food chain. But, the relative contribution of foods to the global burden of infections caused by antibiotic pathogenic bacteria has not yet been determined (Likotrafiti et al., 2018).

The overuse or misapplication of antibiotics in food animals for therapy and prophylaxis of bacterial infections or their use in animal feeds as growth promoters in the processing of food animals, the use of antibiotics as food additives and human bacterial infection treatment leads to the development of antibiotic-resistant pathogenic bacteria (VanBoeckel et al., 2015).

The first global surveillance of animal antibiotic use and produced a reference estimation of its current global importance. It is now generally accepted that more antibiotics are used in food and animal production than human consumption (WHO, 2018).

This inevitably led to the rise of resistant pathogenic bacteria in the gut of animals, and due to their resistance to widely used therapeutic antibiotics, these bacteria can cause severe infections for which therapeutic options are limited. In other words, whenever the non-needed antibiotic is applied to food production, it creates a risk of antibiotic-resistant infections and outbreaks (Christy et al., 2018).

Besides this, AMR bacteria can also reach crops and plants through contaminated manure or sewage water that is used for fertilization and irrigation (Heather, 2014).

In this regard, the current information available from the European Food Safety Authority (EFSA) and the European Center for Disease Prevention and Control (ECDC) provided scientific evidence that there is a connection between the use of antibiotics in animal, crop and food production leads to the occurrence AMR food-borne pathogenic bacteria on raw and ready-to-eat foods. As a result, potentially foodborne infections and outbreaks occur on the general public (European Food Safety Authority/European Centre for Disease Prevention, 2018).

Increasing the resistance of Salmonella and Shigella spp to widely used antibiotics is now a threat to modern medicine and as an emerging public health problem, and they were 100% resistant to amoxicillin, 41% to erythromycin and 35% to amoxicillin-clavulanic (Mengist et al., 2018).

Salmonella, Vibro cholera, E.coli, and Shigella spp were the most frequent and common pathogenic AMR foodborne bacteria that were identified during the outbreak investigation in low-income countries including Ethiopia (Ankita and Sevitha, 2016; Ventola, 2015). Therefore, a better understanding of the prevalence of AMR bacteria along the food chain is urgently needed (CDC, 2019; Founou et al., 2016).

Overuse and misuse of antibiotics are generally associated with the development and spread of resistant bacteria and contribute to the ineffectiveness of treatment and poses a serious risk to public health (Robert wood Johnson foundation, 2015).

On the other hand, antibiotics increase selective pressure in bacterial communities, leading to the death of susceptible bacteria; this raises the percentage of resistant bacteria that continues to grow. resistant bacteria can have a growth advantage and grow faster than susceptible bacteria, even at very low levels of antibiotics (Linus, 2014; Lucy et al., 2015).

AMR bacteria may endanger the Sustainable Development Goals (SDG 1,2,3,6 & 8) and the global response to the ongoing threat of infectious diseases caused by AMR bacteria has placed each country at risk and led to low universal health coverage (Dusan et al., 2016; Gerald et al., 2017).

2.4. AMR bacteria in Ethiopia

In Ethiopia, bacteria isolated from different environmental sources would be resistant to many antibiotics and result in high public health threats and economic loss (Assefa and Girma, 2019).

For instance, Escherichia coli spp mostly leads to severe food poisoning as well as common infections such as acute watery diarrhea, urinary tract infection and meningitis and resistant to ampicillin (100%), sulfamethoxazole-trimethoprim (100%), clindamycin (80%), erythromycin (60%) in Ethiopia (Tadesse et al., 2018).

Table 1: AMR bacterial prevalence in Ethiopia from different sample media by review different kinds of literature

Bacteria	Sample source	Antibiotics and level of resistance (%)	Reference
E. coli	Air samples	Vancomycin (75.3%), cotrimoxazole (84%), gentamicin (78.2%) and ciprofloxacin (82.6%),	(Solomon et al., 2017)
Klebsiella spp	clinical specimens	Penicillin G (81.8%) and cotrimoxazole (81.1%)	(Tsegaye et al., 2019)
Neisseria gonorrhea	clinical specimens	Ciprofloxacin 19% (95% CI: 15.0, 23.0)	(Sisay et al., 2018)
Salmonella, Shigella, and E. coli	effluents of hospitals	Resistant to ≥ 4 antibiotics (>56%)	(Fekadu et al., 2015)
Salmonella, Shigella, and E. coli	hospital wastewater	Resistant to ≥ 3 antibiotics (71.7%)	(Dires et al., 2018)
Salmonella, Shigella, and E. coli	Hands of the food handlers	Resistant to ≥ 3 antibiotics (13.2%)	(Getie et al., 2019)
Staphylococcus spp	Ready-to-eat (RTE) food	Cefoxitin(49.6%), clindamycin (39.3%), tigecycline (27.4%), rifampin (20.5%), tetracycline (17.9%), and erythromycin	Chajęcka-Wierzchowska et al., 2014)

		(8.5%)	
Salmonella	Food	Tetracycline (42.6%), cotrimoxazole (28.6%), and ampicillin (14.3%)	(Ejo et al., 2016)

Look out for these situations, in 2015; the World Health Assembly is implementing a global action plan to combat antimicrobial-resistance infections by proposing the following five goals:

1. To improve AMR awareness and understanding through effective communication, education, and training;
2. To build up the knowledge and evidence base through surveillance and research;
3. Reducing the occurrence of antibiotic-resistant infection by taking appropriate measures such as sanitation, hygiene and infection prevention, and control
4. To maximize the appropriate use of antimicrobial drugs in the health of humans and animals; and
5. Developing the economic case for sustainable investment that takes into account all country's needs and growing investment in new drugs, diagnostic tools, vaccines, and other interventions.

2.5 Conceptual Framework

The conceptual framework develops from reviewing literature and it shows the relation among the occurrences of AMR bacteria on RTF with various contributing factors

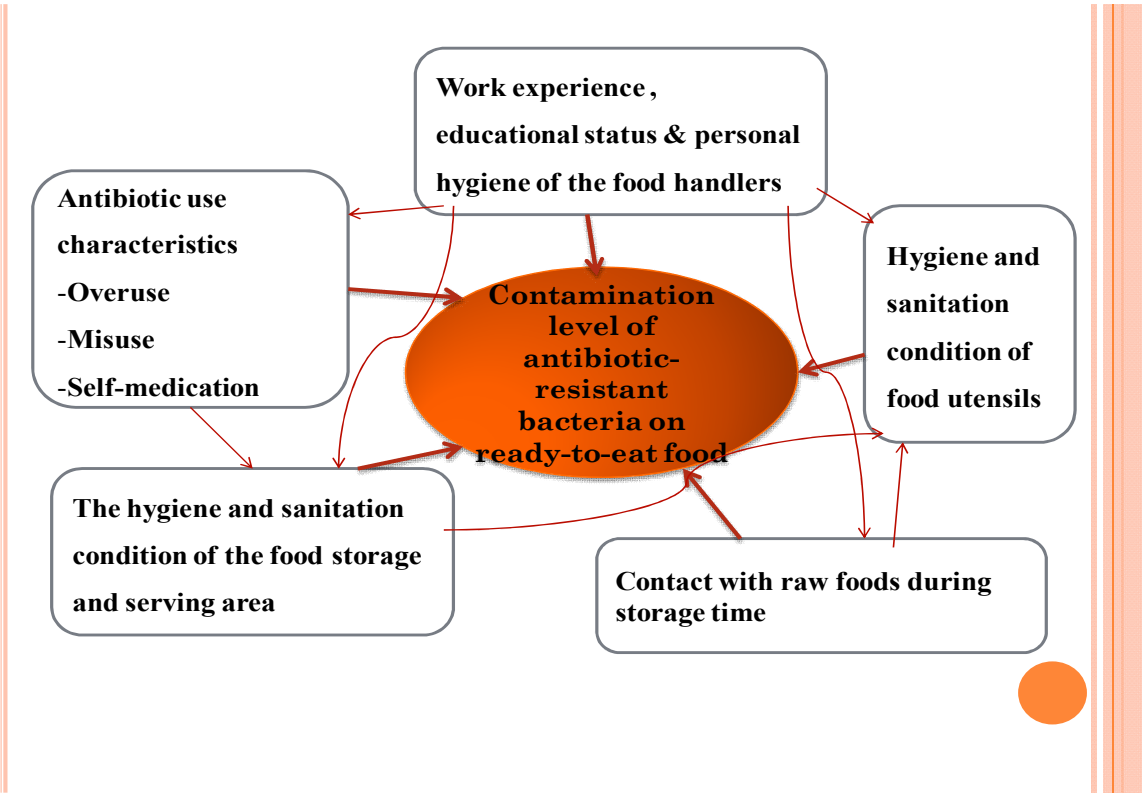

Figure 1:A conceptual framework for the occurrence of antibiotic-resistant bacteria on ready-to- eat food develops by reviewing the literature (Muloi et al., 2018; Tadesse et al., 2018; WHO, 2019).

CHAPTER THREE: OBJECTIVES

3.1 General Objective

To assess the ready-to-eat food contamination with antibiotic-resistant bacteria and associated factors in the Debre Tabor University student cafeteria.

3.2 Specific Objectives

- To isolate the foodborne bacteria on ready-to-eat food
- To test multiple antibiotics- resistant profiles of the isolated foodborne bacteria.
- To determine the ready-to-eat food contamination level with antibiotic-resistant foodborne bacteria
- To identify factors associated with the high contamination of ready-to-eat food with antibiotic-resistant foodborne bacteria.

CHAPTER FOUR: METHODS AND MATERIALS

4.1 Study area

The study was conducted at the Debre Tabor University (DTU), which was established in 2008 G.C, The DTU is a public higher education institution located in the large town of South Gondar, and now it has 14,202 student and1805 staffs and the laboratory investigation was done in the Felege Hiwot comprehensive specialized Hospital.

4.2 Study design and period

A laboratory-based cross-sectional study was carried out during March 2020.

4.3 Sample collection

Samples were collected using the standard set by the United States Environmental Protection Agency sampling standards and District laboratory practices of tropical countries (Monica, 2005, 2006; US-EPA, 2018). Fifty grams of ready-to-eat food (RTF) were collected from the dish. Besides, sixty swab samples were taken from the hand of food handlers and food utensils by using a sterilized cotton swab and work experience, educational status of the food handlers and samples were transported to the laboratory in a cold box with ice-packs immediately after collection for processing and analysis by packed separately and other factors data were collected by using observational checklist and interview questions as shown below.

Figure 2: Sample collection procedures and transport method of the study, March 2020.

4.4 Sample processing techniques for bacterial isolation and susceptibility testing

4.4.1 Sample preparation

The 50g food samples and swab samples were homogenized in to 450ml sterile 0.1 % (w/v) bacteriological peptone in the flask for five minutes.

A 1ml of the homogenized samples were added to 9ml sterilized distilled water and mixed gently by inverting the test tubes several times (Naveena & Joy, 2016).

4.4.2 Foodborne bacterial isolation and identification technique

A 0.1ml of the prepared diluted sample was poured onto Salmonella-Shigella (SS) agar, MacConkey agar plate and Thiosulphate citrate bile and sucrose (TCBS) agar plate for the isolation Salmonella, Shigella spp, Escherichia coli (E.coli) and Vibro cholera respectively and kept the plates in an upright position for few minutes and incubated the plates in an inverted position at 37°C for 18-24 hours. Finally based on the media labels, isolated the specific bacterial species by their differential and selective agar media and colony morphology (Food Standards Australia New Zealand, 2018; Naveena& Joy, 2016).

Figure 3: Isolation of foodborne bacteria in RTF of the DTU student cafeteria, March 2020.

4.4.3 Multiple antibiotic-resistant profile testing

The multiple antibiotic-resistant profiles of all the above isolated foodborne bacteria were carried out on Mueller-Hinton agar with an antibiotic disc using Kirby-Bauer disc diffusion method against 5 currently used antibiotics in Ethiopian healthcare facilities. multiple antibiotic-resistance indexes (MAR) were found out by the formula: MAR index of isolate = No. of antibiotics to which an isolate is resistant/Total no. of antibiotics to which the isolate was exposed, based on the guidelines developed from Clinical and Laboratory Standards Institute of US (Clinical and Laboratory Standards Institute, 2016).

Figure 4: The isolated foodborne bacteria antibiotics sensitivity test of DTU student cafeteria, March 2020.

4.5 Study variables

4.5.1 Dependent variable

Contamination level of RTF with AMR foodborne bacteria

4.5.2 Independent variables

Overuse, misuse and self-medication of antibiotics

Work experience of the food handlers

Educational status of the food handlers

Contact of ready-to-eat food with raw food

Personal hygiene of food handlers

Sanitation conditions of food utensils

4.6 Operational definitions

Food handler - any person who directly handles packaged or unpackaged food, food equipment, and utensils, or food contact surfaces (Meleko et al., 2015).

Inappropriate antibiotic use: included the following parameters: a wrong indication, wrong duration, improper place of administration, use of leftover antibiotics from a family member, and immature discontinuation of antibiotics (Meleko et al., 2015).

Ready-to-eat (RTE) foods- foods that are intended to be eaten without any further process by the final consumer that may eliminate or reduce pathogenic microorganisms that could be present to a safe level (Food Standards Australia New Zealand, 2018).

4.7 Data quality control

Before the actual data collection, training, and discussion with the supervisors, data collectors, and laboratory technician, was undertaken. To keep the quality of the sample, every essential procedure were taken starting from collecting to the analysis of these samples such as sterilization of sampling equipment, utilization of personal protective clothing, glove, cold box to bring and take the sample, proper handling of sterilized materials, safe incubation of samples and use the control (blank) like using of non-inoculated media for samples and antibiotics. The location and the duration of the media in the sampling room, the way of safe transportation, and control cross-contamination, as well as safe analysis in the laboratory were maintained.

4.8 Data management and analysis

The data were coded and entered using Epi info 7 and exported to SPSS version 20.then the Mean prevalence, variability, and linear regression were executed by using SPSS statistical

software version 20. The variances between groups were handled by analysis of variance (ANOVA). Linear regression was conducted to determine the relationship between AMR bacterial contamination of ready-to-eat food with associated factors.

4.9 Ethical considerations

Ethical clearance was obtained from the Institutional Review Board of the Jimma University and an official letter was submitted to the concerned bodies. The concerned bodies were informed to get the assurance of the study and confidentiality was maintained at all levels of the study.

CHAPTER FIVE: RESULTS

5.1 Contamination level of RTF with AMR foodborne bacteria

Prevalence and antibiotic susceptibility patterns of foodborne bacteria were measured by means of taking 30 ready-to-eat foods (RTF) samples, about 12 (43%) (95% CI: 41.2, 46.9%), 10 (36.7%) (95% CI: 33.2, 38.7%) and 06 (20%) (95% CI: 19.2, 26.9%) were culture positive for foodborne E. coli, Salmonella and Shigella spp respectively.

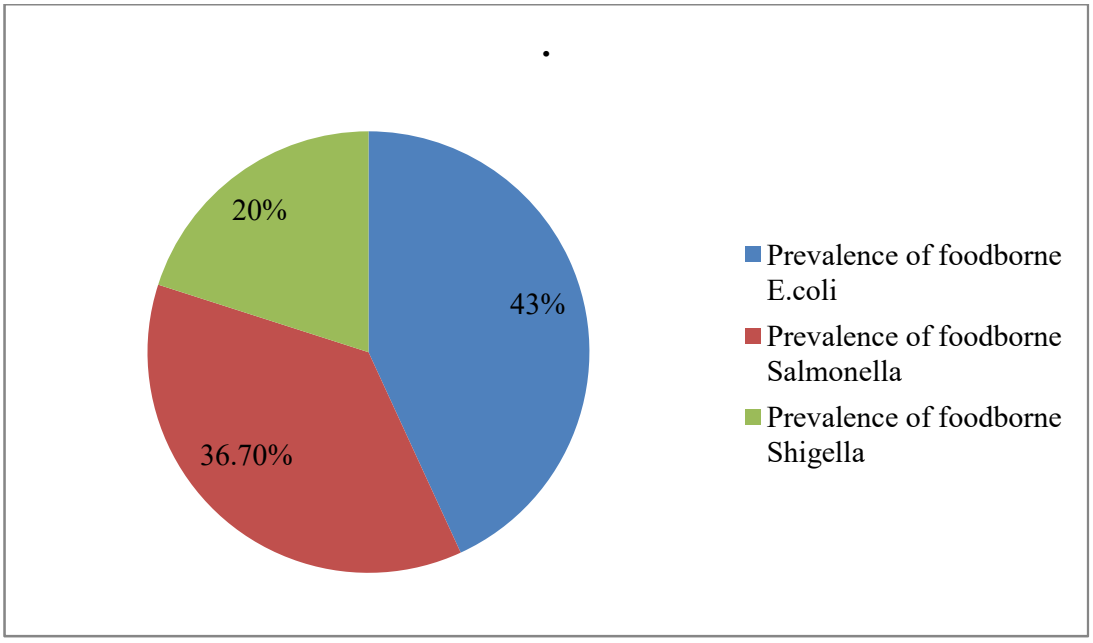

Figure 5: The prevalence of foodborne bacteria on RTF of the DTU student cafeteria, March 2020.

Those isolated foodborne bacteria species antibiotic resistance test was performed using the disc diffusion method against five currently used and prescribed antibiotics in Ethiopian healthcare facilities that were used to treat foodborne bacterial infections. According to the Amhara Regional State dataset report 2019, amoxicillin, cotrimoxazole, doxycycline, ciprofloxacin, and vancomycin were as of now used to treat bacterial infections (ARSHB, 2019). The antibiotic discs of those antibiotics were aseptically impregnated on the agar plates using a sterile forceps and determining the zone of inhibition and matched with the interpretative chart of Clinical and Laboratory Standards Institute (2017) to determine the sensitivity, intermediate and resistance profiles of the isolated foodborne bacteria to the antibiotics used.

In the present study, the isolated E. coli spp showed an antibiotic resistance rate (ARR) of 83.3% to amoxicillin with a mean inhibition zone (MIZ) of 9.2mm (95% CI: 7.4, 10.5), 100% to cotrimoxazole with a MIZ of 7mm (95% CI:6.5, 8.9), and 100% to vancomycin with MIZ of

8mm (95% CI:6, 10) and sensitive to ciprofloxacin (100%) with a MIZ of 23mm (95% CI:18.5, 28.9) and doxycycline (75%) with a MIZ of 28mm (95% CI:25.5, 30).

In this study also, the isolated Salmonella spp showed an ARR of 100% to vancomycin with MIZ of 8mm (95% CI:6, 10), 75% to amoxicillin with a MIZ of 7mm (95% CI:5.5, 8.9), 80% to cotrimoxazole with a MIZ of 8.3mm (95% CI:6.2, 9.4) and sensitive to doxycycline (70%) with a MIZ of 33mm (95% CI:27.5, 34.9), and ciprofloxacin (100%) with a MIZ of 22mm (95% CI:19, 26). Also, an ARR of 75% to doxycycline with a MIZ of 9.5mm (95% CI:8.2, 10.1), 100% to vancomycin with a MIZ of 7mm (95% CI:5.3, 10), 83.3% to amoxicillin with a MIZ of 8.5mm (95% CI:6.4, 9.5), 100% to cotrimoxazole with a MIZ of 9mm (95% CI:8, 10) and sensitive rate of 82.3% to ciprofloxacin with a MIZ of 19mm (95% CI:16.5, 23.2) were confirmed in the isolated Shigella spp.

Table 2: Multidrug resistance levels of the isolated foodborne bacteria on RTF, March 2020.

Foodborne bacterial species	Resistance		Sensitive		Mean MDR rate	MAR Index
	Antibiotics	Overall MIZ	antibiotics	Overall MIZ		
E.coli	Amoxicillin, cotrimoxazole, and vancomycin	8.07mm (95% CI: 6.63, 9.8)	Ciprofloxacin and doxycycline	25.50mm (95% CI: 22, 29.45)	94.43%	0.6
Salmonella	Cotrimoxazole, vancomycin, and amoxicillin	7.77mm (95% CI: 5.90, 9.43)	Ciprofloxacin and doxycycline	27.50mm (95% CI: 23.25,30.45)	85%	0.6
Shigella	Cotrimoxazole, amoxicillin, vancomycin, and doxycycline	8.75mm (95% CI: 6.98, 9.90)	Ciprofloxacin	19mm (95% CI: 16.5, 23.2)	89.58%	0.8

MAR test results of this study revealed that the mean MDR values ≥85% (resistant to 3 or more antibiotics) and MAR index ≥0.6 were observed. According to the interpretative chart of the Clinical and Laboratory Standards Institute of US (2017) and clinical expriences, almost all isolated foodborne bacterial species were MAR and result in a high level of contamination of ready-to-eat food. Therefore, the current study inferred that the ready-to-eat food of the Debre

Tabor University student cafeteria was contaminated with MAR Escherichia coli spp 43% (95% CI: 41.2, 46.9%), Salmonella spp 36.7% (95% CI: 33.2, 38.7%) and Shigella spp 20% (95% CI: 19.2, 26.9%) with an overall multidrug-resistant level of 94.43%, 85% and 89.58% and the multiple antibiotics resistance indexes of 0.6, 0.6 and 0.8 respectively.

5.2 Factors associated with the RTF contamination level with AMR bacteria

5.2.1 Socio-demographic characteristics of the food handlers

Out of thirty food handlers included in the study, about 12(40.00%) of the food handlers had secondary education and 18(60.00%) of the food handlers had above secondary education respectively. Approximately 63.33% of the food handlers had appropriate drug use characteristics, 60% of the food handlers had food handling experience of 2 to 5 years and also about 80% of the food handlers had not taken food safety training.

Table 3: Socio-demographic characteristics of food handlers, March 2020.

Study variables	Category	No	Frequency (%)
Educational status of FHs	Secondary education	12	40
	>secondary education	18	60
Work experience of FHs	< 2 years	11	36.67
	2-5 years	18	60
	>5 years	1	3.33
Food safety training of the FHs	Yes	6	20
	No	24	80
Drug use XXS	Appropriate	19	63.33
	Inappropriate	11	36.67

5.2.2 Prevalence of AMR foodborne bacteria on food utensils

Of the total thirty food utensils swab samples experimental analysis data revealed that, About 07(23.3%) (95% CI, 21.2%, 26.9%), 07(23.3%) (95% CI, 21.2%, 26.9%) and 05 (16.7%) (95% CI, 14.2%, 19.9%) were culture positive for AMR E. coli, Salmonella and Shigella spp respectively (Fig.6).

5.2.2 Prevalence of AMR foodborne bacteria on the hand of food handlers

The thirty hand swab samples of the food handler laboratory analysis information showed that, Almost 10 (33.3%) (95% CI, 31.5%, 36.4%), 9(30%) (95% CI, 29.8%, 36.0%) and 06 (20%)

(95% CI, 19.2%, 26.9%) were culture positive for AMR E. coli, Salmonella and Shigella spp respectively (Fig .6).

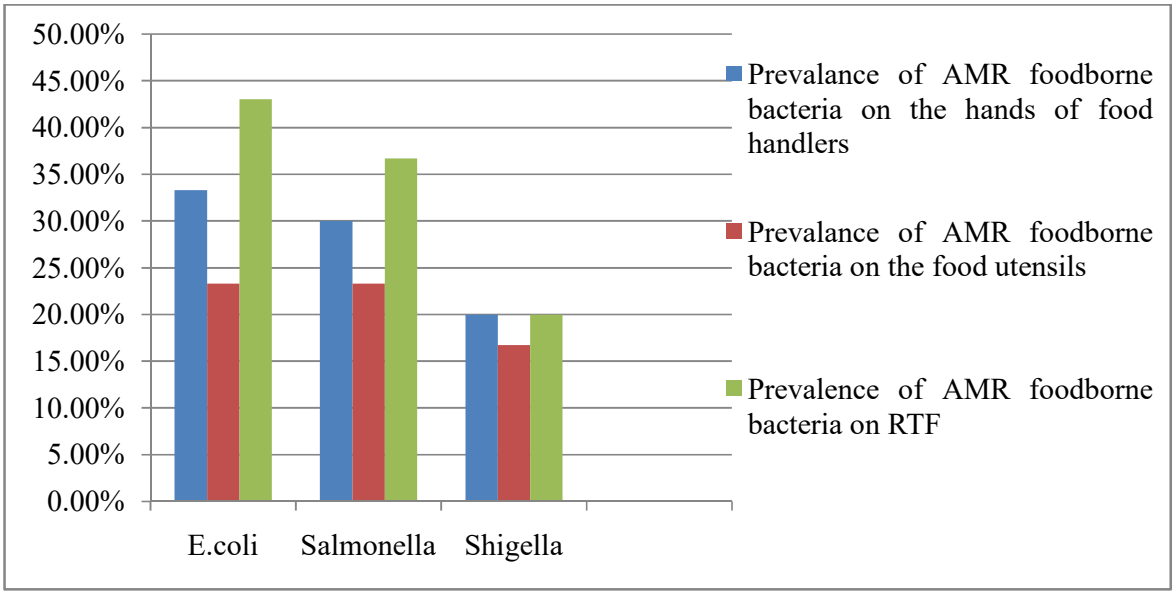

Figure 6: The prevalence of AMR bacteria on RTF, hands, and utensils, March 2020.

5.3. Association of the study variables

In this study, linear regression and Pearson correlation analysis were conducted and presented the outputs on the table below for measuring the presence of association and strength of association.

Table 4: AMR bacterial contamination of RTF with major contributing factors, March 2020.

Study variables			E.coli on RTF		Salmonella on RTF		Shigella on RTF		Pearson correlation coefficient (r)	P-value	Strength of associations
			Yes	No	Yes	No	Yes	No			
Educational status of FHs	Secondary education	12	10	2	7	5	4	8	-0.93	0.00	Strong
	>secondary education	18	2	16	3	15	2	16			
Work experience of FHs	< 2 years	11	10	1	6	5	3	8	- 0.85	0.025	Strong
	2-5 years	18	2	16	4	14	3	15			
	>5 years	1	0	1	0	1	0	1			
Food safety training of the FHs	Yes	6	0	2	0	2	0	2	-0.38	0.038	Moderate
	No	24	12	16	10	18	6	22			
Drug use XXS	Appropriate	19	4	15	3	16	1	18	0.65	0.00	Moderate
	Inappropria	11	8	3	7	4	5	6			

	te										
Bacteria on food utensil			7	23	7	23	5	25	0.98	0.007	Strong
Bacteria on hand of the FHs			9	21	8	22	4	26	0.95	0.000	Strong
Contact b/n raw food and cooked foods	Yes	08	5	3	4	4	3	5	0.07	0.41	No association
	No	22	7	15	6	16	3	19			

Where: XXS- Characteristics FHs- Food Handlers

The contamination level of RTF with AMR foodborne bacteria had statistical significant association with educational status (r = -0.93, P-value = 0.00), food safety training (r = -0.38, P-value = 0.038), work experience (r = -0.85, P-value = 0.025), drug use characteristics (r = 0.65, P-value = 0.00), personal hygiene (r = 0.95, P-value = 0.007) of food handlers, sanitation conditions of food utensils (r = 0.98, P-value = 0.00).

CHAPTER SIX: DISCUSSION

Identification and determination of RTF contamination with AMR foodborne bacteria are very crucial to ensure food safety in University catering services where a comparatively large number of clients served regularly since single contamination may lead to outbreaks (Meleko et al., 2015). The present study attempted to analyze AMR foodborne bacterial contamination rates of the commonly served RTFs in the DTU student cafeteria and to correlate potential associated factors with the extent of AMR bacterial contamination of that RTF.

In this study, AMR foodborne bacterial contamination rates were 43%, 36.7% and 20% of E. coli, Salmonella and Shigella spp with the MDR level of 94.43%, 85% and 89.58% and the multiple antibiotics-resistant indexes of 0.6, 0.8 and 0.8 respectively (Fig.5 and Table 2), from 30 RTF samples. This is higher than study was done in India with the contamination rate of AMR E. coli (42%), Salmonella (9%), and Shigella (3%) (Kaur and Walia, 2020) and the study conducted in Egypt with the contamination rate of AMR Salmonella and E. coli spp were 6.66% and 4.16% respectively (Younis et al., 2019). The difference might be due to the service years of the study area, personal hygiene of the food handlers, food safety awareness of the food handlers, and sanitation condition of the food serving area.

The contamination level of AMR E.coli in the present study is consistent with the study conducted in Mekelle with the contamination level of AMR E.coli spp of 45.35% with MDR level of higher than 65% (Tadesse et al., 2018). However, this is higher than the study conducted in Nigeria with a contamination level of AMR E.coli of 11.1% with a MDR level of >70% (Mamza et al., 2010). The difference might be due to the study method and period, personal hygiene of the food handlers, food safety awareness of the food handlers, and sanitation condition of the food serving area.

The contamination rate of AMR Salmonella spp in the current study are comparable to the study done in Bangladesh with a contamination rate of 30.25% and a MDR level of 72-93% (Mahmud et al., 2016). On the other hand, This is higher than the study conducted in Jigjiga with a contamination rate of 20.8% (Wolde et al., 2016). The difference might be due to the service years of the study area, years of the study, personal hygiene of the food handlers, food safety awareness of the food handlers, and hygiene and sanitation condition of the food serving area.

In this study, the contamination rate of Shigella spp is higher than the review conducted in Ethiopia with an overall contamination rate of 6.6% and a multi-drug resistant (MDR) rate of

86.5% (Hussen et al., 2019). However, the lower contamination rate and MDR level were observed in Pakistan (Rizwan et al., 2018). The difference might be due to the service year of the study area, year of the study, methods, personal hygiene of the food handlers and hygiene and sanitation condition of the food preparation and serving areas.

The contamination level of RTF with AMR foodborne bacteria was strongly predicted by the personal hygiene of the food handlers and sanitation conditions of food utensils (Fig.6 and Table 2). And had a statistically significant association with educational status, food safety training, work experience, and drug use characteristics of food handlers (Table 2). These findings are similar to the study conducted in Italy, approximately 38% of RTE foods were contaminated with AMR bacteria due to poor hygienic processing and handling of foods (p-value < 0.05) (Vincenti et al., 2018).

This finding is also comparable to the study conducted in Dilla, the food contamination level with AMR foodborne Salmonella and Shigella spp were statistically associated with educational status (P-value = 0.04) and service in the year (p-value = 0.021) of the food handlers. And their resistance antibiotics and overall multidrug resistance rate are shown below (Table 5) (Diriba et al., 2020).

Table 5: AMR foodborne Salmonella and Shigella species isolated from food handlers in Dilla University, Southern Ethiopia.

Antibiotics	Resistance pattern	Resistance and sensitivity rate of Salmonella species ($n = 22$)	Resistance and sensitivity rate of Shigella species ($n = 7$)
Amoxicillin	S	6(27%)	1 (14%)
	R	16(73%)	6 (86%)
Ciprofloxacin	S	17 (77%)	6(86%)
	R	5(23%)	1(14%)
Cotrimoxazole	S	7(32%)	7(32%)
	R	15 (68%)	15(68%)
Chloramphenicol	S	6(27%)	3(43%)
	R	16(73%)	4(57%)

The current findings are also consistent with the study conducted in Nepal, a high contamination level of RTF with a MDR foodborne bacteria were associated with non-gloved food handlers ($p \leq 0.05$) and unsanitizing food utensils ($p \leq 0.05$) (Sapkota et al., 2019). And the comparable study was done in Brazil, the contamination of the food with AMR foodborne bacteria usually correlates with inadequate hygiene, inappropriate food handling, and cross-contamination (P-value=0.05) (Lima et al., 2017).

CHAPTER SEVEN: CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

Ready-to-eat food of the Debre Tabor University student cafeteria were contaminated with AMR Escherichia coli spp 43% (95% CI: 41.2, 46.9%), Salmonella spp 36.7% (95% CI: 33.2, 38.7%) and Shigella spp 20% (95% CI: 19.2, 26.9%) with overall multidrug-resistant level of 94.43%, 85% and 89.58% respectively.

The high contamination rate of RTF with AMR foodborne bacteria had a statistically significant association with poor personnel hygiene of the food handlers, work experience of fewer than 5 years of the food handlers, inappropriate drug use characteristics of the food handlers, lack of food safety training of the food handlers, educational status of less than or equal to secondary education of the food handlers and poor sanitary condition of food utensils.

7.2 Recommendations

Therefore, based on the finding obtained the following recommendation was forwarded.

Food handlers should be responsible to wash hands based on WHO handwashing guideline, wear personal protective equipment (PPE), take the antibiotics based on doctor recommendation, keep clean the food preparation and serving areas and properly wash and sanitize the food utensil

University administrations should be responsible to arrange the food safety training program, provide enough personal protective equipment(PPE), strengthen the monitoring and evaluation systems of the sanitation and hygiene status of the food handlers and working environment, provide food safety training for newly recruited food handlers, give in service food safety training for food handlers, provide continuing education for food handlers, take corrective measures immediately if there is any food contamination occurred and make it as a thematic area for any project and research calls.

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ANNEXES

Annex 1. A consent form and observational checklist and interview questions

Dear Sir/madam;

My name is _____and I am from_____. I am conducting data collection on antibiotic- bacteria on ready-to-eat food in the Debre Tabor University student cafeteria.

The study being conducted by Mr. ChalachewYenew from Jimma University.

You are kindly requested to be included in the study which has great importance in improving health. The interview will take a maximum of _____minutes.

No information concerning you as an individual will be passed to another individual or institution. Your participation will be based on your willingness and you have the right not to participate fully or partially. If you agree to be included in the study, I will start my question by asking general identification questions.

1. Agree to participate_____
2. Do not Agree to participate_____

Thank you for your cooperation!!!

Name of data collector ---------- Date ---------- Signature ----------

Name of the supervisor ---------- Date ---------- Signature ----------

Part I: Socio-demographic data assessment tool

1. Educational status of respondent ----------

2. Work experience of respondent_____ _____

Part II: Other associated factors assessment tools.

3. Do you use any food additives for the sampled ready- to- eat food? a) yes ____ b) no ____

4. Have you got any information on antibiotic-resistant foodborne pathogenic bacteria? a) yes ____ b) no ____

5. Do you use any antimicrobials in this sampled ready-to-eat food? a) Yes ____ b) no ____

6. Have you taken any medication this week? a) yes ____ b) no ____

6.1. If yes, for what purpose_____

7. Is there any hand contact of this sampled ready-to-eat food? a) yes ____ b) no ____

8. Is there contact between sampled ready-to-eat food with other raw food in this storage(*please verify*)?a) yes ____ b) no

Annex: 2. Bacterial isolation techniques and procedure

Method

Culture Method

Concepts

Make a Serial dilution by taking a ready-to-eat food and swab samples and diluting them through a series of standard volumes of sterile diluents, e.g. distilled water. Then a small measured volume of each dilution is used to make a series of pour plates.

By diluting the sample in this controlled way it is possible to obtain an incubated plate with easily identifiable and countable colonies of bacteria present in the samples.

Materials

Sterile pipette, Syringe, test tube, distilled water, disinfectants, selective and differential media, and incubator

Procedure

1. Take a sterile pipette.

2. Place the syringe onto the plugged end of the pipette

3. Draw up food and swab samples into 9ml sterilized water.

4. Mixing the sample well and provides an initial dilution of 10^{-1}.

5. Mix the dilution thoroughly, by emptying and filling the pipette several times.

6. Discard this pipette into the pot of disinfectant, but keep the syringe for making the next dilution.

7. Take a new pipette, fit it to the syringe and draw up a 1ml sample of the 10^{-1} dilution and place it in the second tube.

8. Mix as well as before. This gives a 10^{-2} dilution.

9. Discard the pipette in disinfectant.

10. The procedure will be repeated up to 7^{th} test tube with a serial dilution of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} using different sterile pipettes and Discarded 1 ml of the sample from 10^{-7} dilution.

11. Pour 0.l ml volume of each dilution By starting with the highest dilution, the same pipette may be used throughout the prepared agar media.

12. Incubate at 37^{o}c for 18-24 hours

Annex: 3. Antibiotic susceptibility testing technique and procedure

Method

Agar Disc Diffusion

Concepts

Disc Diffusion Techniques for Antibiotic-Susceptibility Testing is a test in which bacteria grown on agar plates (usually Mueller Hinton agar) along with antibiotics-impregnated discs. Inhibition of bacterial growth around the disc is observed (zone of inhibition) which indicates whether a particular antibiotic is inhibitory to the growth of bacterial culture being tested. The size or the diameter of this zone depends on how effective the antibiotic is at stopping the growth of bacteria. The more effective antibiotic will create a larger zone of inhibition while ineffective antibiotics will show the smaller zone of inhibition(>15mm zone of an inhibition-Sensitive, 10-15mm zone of inhibition-Intermediate and < 10mm zone of inhibition-Resistant).

Requirements

Alcohol (70 %**),** Bunsen burner**,** isolated colonies on selective and differential agar,

Mueller Hinton agar, test tube with sterile saline, sterile distilled (control)

Inoculating wire or needle, cotton swab, forceps, antibiotic disc, and Incubator

Procedure

Aseptic technique should be used throughout.

1. Mark and label four sections on the base of the Petri dish, for the three different samples and control (sterile water).

2. Using sterile forceps (flamed with alcohol and cooled) remove one filter paper disc, and place the antibiotic disc on an agar plate.

3. Wash the forceps free of the sample.

4. Repeat for the remaining samples and the control (sterile water).

5. Seal the lid to the base with tape.

6. Invert the plate and incubate at 37°C for 18- 24 hours.

7. Examine the plate (without opening). Measure and record the size of any zones of inhibition around the antibiotic discs.