Antimicrobial-Resistant Pathogens in Food Handlers Serving in Mass Catering Centers

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Research

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Abstract

Background

The prevalence of antimicrobial-resistant pathogens, including foodborne antibiotic-resistant bacteria, is ever increasing. An increase in antimicrobial resistance results in treatment failure and outbreaks. Mass food serving institutions are at a high risk of outbreaks due to the probability of mass infection.

Objective

This study aims to determine the contamination of food handlers with antibiotic-resistant bacteria and its associated factors at Debre Tabor University cafeteria.

Methods

A laboratory-based cross-sectional study was conducted by following standard microbiological methods to isolate and identify foodborne bacteria from the hands of food handlers. The Kirby-Bauer disc diffusion method was used for perform the resistance profiles of the foodborne bacteria that were identified from the hands of the food in March 2020. Thirty samples each from hand and food utensil swabs were collected. Besides, work experience, drug use characteristics, and educational status of the food handler’s data were collected by using an observational checklist and interview questions. Descriptive statistics, correlation, and linear regressions were used to analyze the data.

Results

The result shows the contamination of food handlers with varying pathogenic microbial organisms. The food handlers were mostly contaminated with multiple antibiotics-resistance (MAR) of 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%). The study identifies poor personnel hygiene, lack of food safety training, and lack of sufficient food safety knowledge of the food handlers.

Conclusions

The contamination of food handlers with antimicrobial-resistant microbes at the university cafeteria could indicate the likelihood of the occurrence of foodborne outbreaks. Hence, continuous awareness creation and strict supervision were preventing the contamination of the food and related consequences.

1. Background

According to the World Health Organization, food is a possible route of highly prevalent antimicrobial-resistance pathogens. It can easily be contaminated from farm to fork with those potential antimicrobial-resistance pathogenic microbes (1).
Globally, AMR pathogens pose the most significant public health and economic threats where ten million humans were at risk, with 700,000 deaths per year and 100 trillion USD of economic loss by 2050. Hence, these figures are assumed to increase significantly if preventive measures could not be done (2).

In Africa, most pathogens are 50 percent to 100 percent resistant to widely used antibiotics and results in more common treatment failures, increased morbidity and mortality, chronic infections, increased infant and child deaths, and other worsening conditions (3). These threats may occur due to weak antibiotics regulatory capacity, circulation of substandard/counterfeit antibiotics, lack of antimicrobial surveillance strategies, weak laboratory capacity for AMR testing, lack of essential laboratory reagents for monitoring and evaluation, and limited quality assurance (4,5).

The increasing occurrence of AMR pathogens and their threats was a concern of the high and low-income countries (6).

The widespread emergence of AMR pathogens has become one of the most serious challenges in Ethiopia due to antibiotic-drug misuse, drug prescription without susceptibility test, self-medication, and a long stay in the hospital environment (7).

Some experimental investigation and surveillance in Ethiopia, E. coli, Shigella, and Salmonella species showed a high level of resistance to frequently recommended antibiotics (8).

The existence of AMR pathogens on food handlers 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 (9).

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 on food handlers or food environments resulting in their presence of both in raw and cooked food at the consumption stage in any mass catering center (10,11). Assessment of the level of AMR contamination and contamination source identification is highly relevant for policy intervention. Therefore, this study aimed to assess the contamination of food handlers with AMR bacteria and associated factors in the student cafeteria of Debre Tabor University.

2. Methods

2.1 Study design and period

A laboratory-based cross-sectional study was carried out during the month from March-April 2020 at Debre Tabor University.

2.2 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 (12,13). Sixty swab samples were taken from both hands and food utensil swabs using a sterilized cotton swab. Additional data were collected on the food handlers; work experience, educational status, and other factors associated with food safety training of the food handlers by using an observational checklist, and interview questions were conducted among the sampled food servers. Samples were transported to the laboratory in a cold box with ice-packs immediately after collection for processing and analysis by packed separately.

2.3 Sample processing techniques of bacterial isolation and susceptibility testing

2.3.1 Sample preparation

The swab samples were homogenized with sterile 9ml of 0.1 % (w/v) bacteriological peptone in the flask for five minutes(14).

2.3.2 Foodborne bacterial isolation and identification technique

A 0.1ml of the prepared diluted sample was directly inoculated on differential and selective agar media after enrichment with Selenite cystine broth and incubated at 37 °C for 18–24 hours. After incubation, the isolates were determined by the following cultural characteristics, colony morphology, and their biochemical results such as hydrogen sulfide production, indole production, triple sugar iron and motility in sulfide-Indole-motility medium, and lysine decarboxylase.

2.3.3 Multiple antibiotic-resistant profile testing

The multiple antibiotic-resistant profiles of the isolated foodborne bacteria were carried out on Mueller-Hinton agar with an antibiotic disc using the Kirby-Bauer disc diffusion method with 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 by Clinical and Laboratory Standards Institute of US (15).

2.4 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 procedures were taken starting from collecting to the analysis of these samples such as sterilization of sampling
equipment, utilization of personal protective clothing, gloves, 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 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.

2.5 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 bacterial prevalence in food handlers with associated factors in mass catering center.

3. Results

3.1 Contamination level AMR foodborne bacteria on the hands of food handlers

The present study analyzed AMR bacterial contamination rates among permanent food handlers (excluded outsource food handlers) in the DTU catering service and contributing factors to AMR bacterial contamination.

In this study, foodborne bacterial species isolation and antimicrobial resistance test was performed using standard culture and Kirby-Bauer disc diffusion method, respectively.

One way ANOVA was applied to obtain Multidrug-Resistant Rate (MDR), overall mean inhibition zone (MIZ), and multiple antimicrobial-resistance (MAR) index within the groups see the output below in Tables 1 and 2).

Table 1: Multidrug resistance levels of foodborne bacteria isolated from food handlers of DTU, March 2020.
### MAR Test Results

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

MAR test results of this study revealed that 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 experiences, almost all isolated foodborne bacterial species were MAR and resulted in a high level of contamination of the food.

Therefore, the current study inferred that the food handlers of the Debre Tabor University catering service were contaminated with MAR *E. coli* 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 MDR level of 94.43%, 85% and 89.58% and the MAR indexes of 0.6, 0.6 and 0.8, respectively (Table 1).

### 3.2 Factors associated with food handlers contamination level with AMR bacteria

The prevalence of AMR foodborne bacteria on the hands of the food handlers of this study was strongly predicted by the sanitation conditions of food utensils. Besides, educational status, food safety training, work experience, and drug use characteristics of food handlers were significantly associated with the high prevalence of AMR (Table 2).

Table 2: AMR contamination level of food handler with critical contributing factors, March 2020.
| Study variables                        | E.coli | Salmonella | Shigella | PCC  | P-value |
|---------------------------------------|--------|------------|----------|------|---------|
|                                       | Yes    | No         | Yes      | No   | Yes     | No     |
| Educational status of FHs             |        |            |          |      |         |        |
| Secondary education                   |        |            |          |      |         |        |
|                                       | 12     | 10         | 2        | 7    | 5       | 4      |
| >secondary education                  | 18     | 2          | 16       | 3    | 15      | 2      |
|                                       |        |            |          |      |         |        |
| Work experience of FHs                |        |            |          |      |         |        |
| < 2 years                             |        |            |          |      |         |        |
|                                       | 11     | 10         | 1        | 6    | 5       | 3      |
| 2-5 years                             | 18     | 2          | 16       | 4    | 14      | 3      |
| >5 years                              | 1      | 0          | 1        | 0    | 1       | 0      |
|                                       |        |            |          |      |         |        |
| Food safety training of the FHs       |        |            |          |      |         |        |
| Yes                                   |        |            |          |      |         |        |
|                                       | 6      | 0          | 2        | 0    | 2       | 0      |
| No                                    | 24     | 12         | 16       | 10   | 18      | 6      |
|                                       |        |            |          |      |         |        |
| Drug use CIX                          |        |            |          |      |         |        |
| Rational                              |        |            |          |      |         |        |
|                                       | 19     | 4          | 15       | 3    | 16      | 1      |
| Irrational                            | 11     | 8          | 3        | 7    | 4       | 5      |
|                                       |        |            |          |      |         |        |
| Bacteria on food utensil              |        |            |          |      |         |        |
|                                       | 7      | 23         | 7        | 23   | 5       | 25     |
| FHSs=food handlers, CIX = characteristics, and PCC = Pearson correlation coefficient

4. Discussion

Identification and determination of the extent of AMR contamination and key contributing factors of the food handlers are very crucial to ensure food safety in mass catering services, such as University, Military, and other mass catering centers to control communicable disease outbreaks (16).

The lower findings were done in India with the contamination rates of AMR E. coli (42%), Salmonella (9%), and Shigella (3%) and Egypt with the contamination rate of AMR Salmonella spp and E. coli were 8 (6.66%) and 5 (4.16%), respectively (17). The difference might be due to 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 an MDR level of >65% (18). However, this is higher than the study conducted in Nigeria with a contamination level of AMR E.coli of 11.1% with an MDR level of >70% (19). The difference might be due to rampant irrational drug use in Ethiopia, bad personal hygiene of the food handlers, food safety awareness of the food handlers, and sanitation condition of the food serving area (20,21).
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 an MDR level of 72-93% (22).

On the other hand, this is higher than the study conducted in Jigjiga with a contamination rate of 20.8% (23,24). The difference might be due to the service year of the study area, year 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 AMR Shigella spp is higher than the review conducted in Ethiopia with an overall contamination rate of 6.6% and a multidrug resistant (MDR) rate of 86.5% (25). However, the lower contamination rate and MDR level were observed in Pakistan (26). 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.

Similar findings have been reported by a study conducted in Italy. In their study, approximately 38% of RTE foods were contaminated with AMR bacteria due to poor hygienic processing and handling of foods (27). Our findings are also consistent with the study conducted in Nepal, where a high prevalence of MDR foodborne bacteria was associated with non-gloved food handlers and unsanitized food utensils (28). Moreover, the comparative study was done in Brazil also indicated that the prevalence of foodborne bacteria usually correlates with inadequate hygiene, inappropriate food handling, and cross-contamination (29).

The level of food handler education was significant to the contamination level of AMR pathogenic bacteria, where education is vital for behavioral changes to practice hygiene. The present finding is also comparable to the study conducted in Dilla, where the food contamination levels with AMR foodborne Salmonella, and Shigella spp were statistically associated with the educational status and service year of the food handlers (30).

5. Conclusions

The food handler of the Debre Tabor University catering center was contaminated with AMR E. coli, Salmonella, and Shigella spp with an overall multidrug-resistance level of 94.4%, 85%, and 89.58% and multiple antibiotic-resistance (MAR) index of 0.6, 0.6, and 0.8, respectively. The high contamination rate of food handlers with AMR foodborne bacteria is associated with short-time (5 years) work experience as a food handler, irrational drug use, lack of food safety training for the food handlers, educational status, and poor sanitary condition of food utensils.

The finding suggests the importance of food safety training for food handlers and strict follow-up of the implementation of acceptable hygienic practices might improve food safety in the mass catering center. Besides, minimizing irrational drug use could also help to reduce AMR in food and the environment.

6. Abbreviations
7. Declarations

Ethics Approval and Consent to Participate

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. Verbal consent was obtained from all participants and the Institutional Review Board of the Jimma University approved it with Ethical approval of Research protocol letter with its reference number IRB00010/2020.

Consent to Publish: Not applicable

Availability of data and materials: All data generated and analyzed during this study are included in the manuscript.

Competing interests: The authors declare that they have no competing interests.

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Authors’ contribution

FT was actively involved in the conception of research issues and development of research proposals, and CY was actively working on supervision, data analysis, writing a research report, and was a major contributor in writing the final manuscript. All authors read and approved the final manuscript.

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References
1. WHO. Who estimates of the global burden of foodborne diseases. WHO Library Cataloguing. Switzerland; 2015.
2. Viens. Is Antimicrobial Resistance a Slowly Emerging Disaster? AMR: Challenge, Threat. Public Health Ethics. 2015;8(3):255–65.
3. Ampaire et al. AMR in East Africa. Afr J Lab Med. 2016;5(1).
4. Ndihokubwayo et al. Antimicrobial resistance in the African Region: Issues, challenges, and actions proposed. WHO, Reg Off Africa. 2013;(16).
5. Founou et al. Antibiotic Resistance in Food Animals in Africa. Microb drug-resistant. 2018;24(5).
6. Aastha et al. Global Contributors to Antibiotic Resistance. JGlobal Infect Dis. 2019;11(3).
7. Tamiru et al. patterns of researches done on antimicrobial resistance in Ethiopia. Indo Am J Pharm Res. 2017;7(09).
8. Moges et al. The growing challenges of antibacterial drug resistance in Ethiopia. Elsevier J Antimicrob Resist. 2014;2(3):148–54.
9. Levy. Food animals and antimicrobials: Impacts on human health. Clin Microbiol Rev. 2011;24(4):718–33.
10. Taban. A State-of-Art Review on Multi-Drug Resistant Pathogens in Foods of Animal Origin: Risk Factors and Mitigation Strategies. Front Microbiol. 2019;10.
11. Verraes et al. Antimicrobial Resistance in the Food Chain: A Review. Int J Environ Res Public Heal. 2013;10:2643–69.
12. US-EPA. Sampling, Laboratory, and Data Considerations for Microbial Data Collected in the Field. Off Res Dev Natl Homel Secur Res Cent. 2018;
13. Monica. District Practice in LaboratoryTropical Countries Part 2. Vol. 1, Cambridge University Press. 2006. 53 p.
14. Naveena & Joy. Microbiology laboratory manual. Pineapple Res Stn. 2016;
15. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing Supplement M100S. 26th ed. 2016. 1–256 p.
16. Meleko et al. Assessment of the Sanitary Conditions of Catering Establishments and Food Safety Knowledge and Practices of Food Handlers in Addis Ababa University Students ‘ Cafeteria. Sci J Public Heal. 2015;5(3):733–43.
17. Younis R, Nasef S, Salem W. Detection of Multi-Drug Resistant Food-borne Bacteria in Ready-to-Eat Meat Products in Luxor City, Egypt. SVU-International J Vet Sci. 2019;2(1):20–35.
18. Tadesse et al. Antimicrobial Resistance Profile of E. Coli Isolate from Raw Cow Milk and Fresh Fruit Juice in Mekelle, Tigray, Ethiopia. Vet Med Int. 2018;2018.
19. Mamza SA, Egwu GO, Mshelia GD. aureus isolated from chickens in Nigeria. 2010;46(2):155–66.
20. Biswas S, Parvez MAK, Shafiquzzaman M, Nahar S, Rahman MN. Isolation and characterization of Escherichia coli in ready-to-eat foods vended in Islamic University, Kushtia. J Bio-Science. 2010;18(1):99–103.
21. Assefa et al. Bacterial Hand Contamination and Associated Factors among Food Handlers Working in the Student Cafeterias of Jimma. Community Med Heal Educ. 2015;5(2).

22. Mahmud T, Hassan MM, Alam M, Khan MM, Bari MS, Islam A. Prevalence and multidrug-resistant pattern of Salmonella from the eggs and egg-storing trays of retail markets of Bangladesh. Int J One Heal. 2016;2(March):7–11.

23. Wolde et al. Prevalence and antimicrobial susceptibility profile of Salmonella species from ready-to-eat foods from catering establishments in Jigjiga City, Ethiopia. African J Microbiol. 2016;10(37):1555–60.

24. Mama M, Alemu G. Prevalence, antimicrobial susceptibility patterns and associated risk factors of Shigella and Salmonella among food handlers in Arba Minch University, South. BMC Infect Dis [Internet]. 2016;1–7. Available from: http://dx.doi.org/10.1186/s12879-016-2035-8

25. Hussen et al. Prevalence of Shigella species and its drug resistance pattern in Ethiopia: a systematic review and meta-analysis. Ann Clin Microbiol Antimicrob. 2019;18(22):1–11.

26. Rizwan M, Naeem M, Pokryshko O. Isolation & Identification of Shigella species from food and water samples of Quetta, Pakistan. Pure Appl Biol. 2018;7(1).

27. Vincenti S, Raponi M, Sezzatini R, Giubbini G, Laurenti P. Enterobacteriaceae Antibiotic Resistance in Ready-to-Eat Foods Collected from Hospital and Community Canteens: Analysis of Prevalence. J Food Prot. 2018 Mar;81(3):424–9.

28. Sapkota S, Adhikari S, Khadka S, Adhikari M, Kandel H, Pathak S, et al. Multi-drug resistant extended-spectrum beta-lactamase producing E. coli and Salmonella on raw vegetable salads served at hotels and restaurants in Bharatpur, Nepal. BMC Res Notes. 2019;12(1):1–13.

29. Lima CM, Souza IEGL, dos Santos Alves T, Leite CC, Evangelista-Barreto NS, de Castro Almeida RC. Antimicrobial resistance in diarrheagenic Escherichia coli from ready-to-eat foods. Vol. 54, Journal of Food Science and Technology. 2017. p. 3612–9.

30. Diriba K, Awulachew E, Ashuro Z. Prevalence and Antimicrobial Resistance Pattern of Salmonella, Shigella, and Intestinal Parasites and Associated Factor among Food Handlers in Dilla University Student Cafeteria, Dilla, Ethiopia. Int J Microbiol. 2020;2020.