

Occurrence of drug-resistant enteric bacteria and associated factors among food handlers of the Mass Catering Center: A laboratory-based cross-sectional study

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Abstract

This study aimed to determine antimicrobial-resistance (AMR) enteric bacterial load and associated factors among Food Handlers (FHs) of Mass Catering Center (MCC), Ethiopia. From January to June 2020, a laboratory-based cross-sectional study was conducted using the standard Swab microbiological analysis method and the Kirby-Bauer disc diffusion method on 160 randomly selected FHs and food serving areas and analyzed with RStudio-1.2.5033 for contamination, resistance level, and source determination. The contamination of AMR *Escherichia coli* was 33.3% (95% CI, 31.5%, 36.4%), *Salmonella* 30% (95% CI, 29.8%, 36.0%) and *Shigella* 20% (95% CI, 19.2%, 26.9%) among the hand of FHs serving in MCC. The contamination was more likely associated with low work experience [AOR, 1.42 (95% CI: 1.22-1.87)], a low educational level [AOR, 1.62 (95% CI: 1.52-189)], irrational drug use characteristics [AOR, 1.75 (95% CI: 1.64-2.00)], lack of sufficient food safety knowledge [AOR, 1.52 (95% CI: 1.32-1.67)] of the FHs and the sanitary condition of the food serving area [AOR: 1.98 (95% CI, 1.45, 2.53)]. The contamination of the hand of FHs serving in the MCC with AMR enteric FBB at the University cafeteria could indicate the likelihood of the occurrence of foodborne outbreaks.

Introduction

According to the World Health Organization (WHO), food handlers (FHs) may be a possible source of highly prevalent enteric antimicrobial-resistance (AMR) pathogens and results in a high burden of foodborne diseases (WHO, 2012).

In some experimental investigations and surveillance in Ethiopia, the common enteric bacteria such as *Escherichia coli* (*E. coli*), *Shigella*, and *Salmonella* species showed a high level of resistance to frequently recommended antibiotics such as Vancomycin (VA); Cotrimoxazole (COT), Amoxicillin (AMX) and were commonly isolated on the FHs (Moges *et al.*, 2014).

The development of AMR pathogens on the hands of FHs is becoming an ever more community health problem worldwide due to the overuse of antibiotics for food additives, food preservation, and their medication (Marshall and Levy, 2011).

Literature indicates that FHs play a significant role in the transmission of AMR pathogens to customers (students and the general population) and result in severe AMR bacterial infections as a result of inadequate screening of FHs, lack of training for FHs on food handling and hand hygiene practices, and irregular monitoring of food handling practices. Consequently, assessing the level of AMR contamination in enteric FBB and identifying the source of contamination are crucial for mitigating risk (Mengist *et al.*, 2018).

Besides, the MCC centers such as Universities, Hotels, *etc.* are ever-increasing. However, food safety issues related to AMR enteric FBB in the MCC of Ethiopia are not well understood and have received little attention. Therefore, this study aimed to assess the contamination of the AMR enteric bacteria and associated factors among FHs in the case of Debre Tabor University (DTU) MCC, Ethiopia.

Materials and methods

Study design, period, and site

A laboratory-based cross-sectional study was carried out from January through June 2020 at DTU MCC, Ethiopia among 160 FHs swabs and 160 swabs for food serving area.

Sample size determination

A total of 160 randomly selected FHs and food serving area each swab samples were taken within 3 visits per day by using equations derived from the WHO and United Nations International Children's

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Emergency Fund and taken a 3 visits average. WHO and United Nations International Children's Emergency Fund were given a sufficient sample size by considering the type of samples (laboratory-based samples), cost, and time of the laboratory-based study (WHO and UNICEF, 2012).

Sample collection

Swab sample collection

The surface swab technique was used as described in the Compendium of Methods for Microbiological Examination (Speck, 2015). The sampling procedure was performed

med by swabbing a delimited area of 100 cm² from the food serving area and a Palm swab of the FHs. A sterile polypropylene template was used to sample each 100 cm² surface. The wetted swab head was rubbed slowly in two directions at right angles to each other. The area was swabbed for approximately 20 seconds (NSW Government Food Authority, 2020). Thereafter, all swab samples were placed in an ice-cooled box and transported to the laboratory for analysis within 4 hours of collection withholding temperature of <4°C immediately to Bahir Dar Institute of Technology, Faculty of Chemical and Food Engineering, Food Quality and Safety Laboratory within 2 hours of sample collection with the support of two laboratory personnel from Food Microbiology. Additional data collected on the FHs; work experience, educational status, and other factors associated with food safety training of the FHs by using an observational checklist and interview questions were conducted.

Exclusion and inclusion criteria

All FHs serving in the MCC met the inclusion criteria.

FHs who had taken drugs and were unwell during the trial period were excluded as exclusion criteria.

Laboratory analysis of FBB isolation and AMR testing

FBB isolation and identification technique: *E. coli* isolation procedures

To test for the presence of *E. coli*, the swab samples were homogenized with sterile 9 ml of 0.1% (w/v) bacteriological peptone water (BPW) in the flask for five minutes.

MacConkey Agar (Oxoid) and Xylose Lysine Desoxycholate (XLD) medium (Oxoid) were used for plating purpose. A loopful of culture from each selective enrichment broth was streaked separately on to each of the solid medium and incubated at 37°C for 18-24 hours.

Based on their characteristic appearance, colonies suspected for *E. coli* were picked from each selective medium and further purified and tested biochemically. Un-inoculated culture plates were incubated to check for sterility of the solid media.

Test portion, 50g+ modified tryptone soya broth supplemented with Novobiocin and the broth should be pre-warmed to 41.5°C before use. Homogenization and incubate at 41.5°C at 6 hours. Capture of *E. coli* by the use of immunomagnetic beads. Re-suspend in 0.1 ml sterile wash-buffer. Inoculation of 50µl immunomagnetic beads on selective media (CT-SMAC and CR-SMAC) to obtain presumptive

colonies. Incubate at 37°C for 18-24 hours.

Isolate five presumptive sorbitol negative colonies from each agar plate and inoculate onto Nutrient agar plates. Incubate at 37°C for 18-24 hours. Confirm by indole test or biochemical identification kits and serological agglutination with *E. coli* antiserum (ISO, 2001).

Isolation and characterization of *Salmonella* and *Shigella*

To test for the presence of *Salmonella* and *Shigella*. The swab samples were homogenized with sterile 9 ml of 0.1% (w/v) bacteriological peptone water (BPW) in the flask for five minutes. From this homogenized sample 10 ml was transferred to 50 ml Tryptone Soya Broth (TSB) and incubated at 37°C for 18-24 hours for the metabolic recovery and proliferation of cells which could have been injured during processing or to bring the number of target organisms to a detectable level.

The following broths were employed for secondary enrichment: Selenite Broth Base (SBB) (Oxoid), Mannitol Selenite Broth Base (MSBB) (Oxoid) both supplemented with sodium biselenite, selenite cystin broth base (SCBB), Tetrathionate Base (TBB) (Oxoid) supplemented with iodide solution (20 ml/l). The selective property of these broths lies in their ability to inhibit non-targeted microorganisms like Gram-positive bacteria and coliforms and permit the rapid multiplication of *Salmonella* and *Shigella*.

After pre-enrichment in Tryptone Soya Broth (TSB), 1 ml of culture was transferred into separate tubes each containing 10 ml of MSBB, TBB, SCBB and SBB. TBB was incubated at 43°C for 48 hours in water bath and the remaining were incubated at 37°C for 24 hours.

Salmonella-Shigella (SS) Agar (Oxoid) and Xylose Lysine Desoxycholate (XLD) medium (Oxoid) were used for plating purpose. A loopful of culture from each selective enrichment broth was streaked separately on to each of the solid medium and incubated at 37°C for 18-24 hours. Based on their characteristic appearance, colonies suspected for *Salmonella* were picked from each selective medium and further purified and tested biochemically. Un-inoculated culture plates were incubated to check for sterility of the solid media.

The growth of *Salmonella* and *Shigella* species was detected by their characteristic appearance on MAC (NLF, smooth, colorless colonies, sometimes with black centered) and XLD agar (small red colonies and black-centered colonies). Confirmatory identification was done by the pattern of biochemical reactions using a standard bac-

terial identification system (Gebremichael *et al.*, 2018).

Multiple antibiotic resistant (MDR) profile testing

The slanted cultures were sub-cultured and purified. The pure colonies were inoculated into Nutrient Broth and incubated at 37°C for 18-24 hours. After incubation, the turbidity of the culture was adjusted to 0.5 McFarland Standard to bring the cell density to approximately 10⁷-10⁸cfu/ml. The 0.5 McFarland turbidity standard was prepared by mixing 0.05 ml BaCl₂ (1%) with 9.95 ml H₂SO₄ (1%). Muller-Hinton (MH) (Oxoid) plates were prepared and warmed to ambient temperature for plating. A sterile cotton swab was dipped into the standardized suspension. The culture was spread evenly over the entire surface of the Muller-Hinton agar plates by wabbing in three directions at 90° of each spreading. The plates were allowed to dry before applying antimicrobial discs. The following standard and Oxoid drug discs were used: Vancomycin (VA) disk of 30µg oxide; Cotrimoxazole (COT) disk of 25µg oxide; Ciproflaxicillin (CIP) disk of 5µg oxide; Doxycycline (DC) of 30µg, Amoxicillin (AMX) disk of 10µg oxide, Erythromycin (ERYC) disk of 15µg oxide, Ampicillin (AMP) disk of 10µg oxide, Ceftriaxone (CRO) disk of 30µg oxide, Chloramphenicol (CHL) disk of 30µg oxide, Penicillin (PEN) of 10µg oxide, Novobiocin (NB) disk of 30µg oxide, Cloxacillin (CLOXA) disk of 1µg oxide, Cefalotin (CET) disk of 30µg oxide, Gentamicin (GEN) disk of 10µg oxide, that were commonly used and clinically important antibiotics in Ethiopian healthcare facilities. After incubation at 37°C for 18 to 24 hr, inhibition zones were measured and scored as susceptible, intermediate, or resistant and 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 the Clinical and Laboratory Standards Institute of US (CLSI, 2017). The *E. coli* ATCC 25922, *Salmonella ser. Choleraesuis* ATCC 10708 and *Shigella flexneri* ATCC 12022 were used as reference strains for antibiotic disk control (Clinical and Laboratory Standards Institute, 2016).

Data quality control

Triplicate samples for identification of microbes due to the study conducted using the Culture Method rather than the Molecular technique and duplicate samples for AMR test because Culture is a golden

for it were used. Information on each sampling site and identification of the sampling locations were done by Global Positioning System (GPS). To check the sterility of the prepared media, 5% of the prepared batch of media was incubated overnight and checked for microbial growth in the media and reference strains also used.

Data management and analysis

The data were coded and entered using Epi info 7 and exported to RStudio-1.2.5033 for analysis. The data were coded and entered using Epi info 7 and exported to Stata version 14.1. Stata 14.1 software was used for data management and further analysis.

Descriptive statistics were employed to examine the prevalence, rate, comparability of results, and cumulative and summary of AMR patterns of different enteric bacteria with sampling weighting for the laboratory data.

The 95% confidence interval was used for considering statistical significance and multiple logistic regression models were used to show the relationship between the prevalence rates of AMR FBB with the associated factors.

Results

Socio-demographic characteristics of the FHs serving in MCC

Out of 160, FHs included in the study, about 64 (40.00%) of the FHs had secondary education

and 96 (60.00%) of the FHs had above secondary education respectively. Approximately 63.33% of the FHs had rational drug use characteristics (not misuse, not overuse, not sharing the drug, properly dispose of the expired drugs, take the drug in the appropriate place and take doctor recommendation), 60% of the FHs had food handling experience of 2 to 5 years and also about 80% of the FHs had not taken food safety training (Table 1).

Contamination level AMRFBB on the hands of FHs serving in MCC

About 33.3% (95% CI, 31.5%, 6.4%), 30% (95% CI, 29.8%, 36.0%) and 20% (95% CI, 19.2%, 26.9%) were culture positive for AMR *E. coli*, *Salmonella* and *Shigella* spp with the MAR (resistance to three or more antibiotics) indexes of 0.57, 0.57 and 0.64, among 160 hand swab samples of the FHs respectively. 57% of isolated *E. coli* resistance to three or more antibiotics, 57% of isolated *Salmonella* resistance to three or more antibiotics, 64% of isolated *Shigella* resistance to three or more antibiotics and the isolates were resistant to AMX, COT, VA, CLOXA, CRO, ERYC, CET & CHL; none was resistant to CIP, AMP, PEN, GEN and NB (Table 2).

Factors associated with contamination of the hands of FHs with AMR enteric FBB

The contamination level of the hand of FHs serving in the MCC with AMR enteric FBB had more likely associated with low work experience (AOR: 1.42 (95% CI: 1.22-1.87), a low education level (AOR: 1.62(95% CI: 1.52-189), irrational drug use characteristics (AOR: 1.75(95% CI: 1.64-2.00), the sanitary condition of the food serving area (AOR:1.98 (95% CI, 1.45, 2.53) and lack of sufficient food safety knowledge (AOR: 1.52(95% CI: 1.32-1.67) of the hands of FHs. Low educational status,

lack of sufficient food safety training, low work experience, and irrational drug use characteristics of FHs were more likely associated with a higher prevalence of AMR FBB on the hands of the FHs. The isolated value of *E.coli*, *Salmonella* and *Shigella* and AOR indicates sanitary condition of the food serving area is major contributor for prevalence of AMR FBB on the hands of the FHs beyond educational status and work experience (Table 3).

Discussion

Identification and determination of the extent of AMR contamination and key contributing factors of the FHs serving in MCC are very crucial to ensure food safety in MCC, such as Universities, Military, and other MCCs to control communicable disease outbreaks (Meleko *et al.*, 2015). This study aimed at determining AMR enteric bacterial load and associated factors among FHs of MCC, Ethiopia.

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 (Younis *et al.*, 2019). The difference might be due to the study area, personal hygiene of the FHs, food safety awareness of the FHs, and sanitation condition of the food

Table 1. Socio-demographic characteristics of FHs of DTU MCC.

Study variables	Category	Frequency	%
Educational status	Secondary education	64	40
	>Secondary education	96	60
Work experience	< 2 years	59	36.67
	2-5 years	96	60
	>5 years	5	3.33
Food safety training	Yes	32	20
	No	128	80
Drug use characteristics	Rational	101	63.33
	Irrational	59	36.67

Table 2. The MDR levels of enteric FBB isolated from the hand of FHs within different antimicrobials of DTU MCC.

FBB species	Resistance		Sensitive		MAR index
	Antibiotics	MIZ	Antibiotics	MIZ	
<i>E.coli</i>	AMX, COT, VA, CLOXA, CRO, ERYC, CET & CHL	8.07 mm (95% CI: 6.63, 9.8)	CIP, AMP, PEN, GENNB & DC (95% CI: 22, 29.45)	25.50mm	0.57
<i>Salmonella</i>	AMX, COT, VA, CLOXA, CRO, ERYC, CET & CHL	7.77 mm (95% CI: 5.90, 9.43)	CIP, AMP, PEN, GENNB & DC (95% CI: 23.25, 30.45)	27.50mm	0.57
<i>Shigella</i>	AMX, COT, VA, CLOXA, CRO, ERYC, CET, CHL, & DC	8.75 mm (95% CI: 6.98, 9.90)	CIP, AMP, PEN, GEN & NB	19mm (95% CI: 16.5, 23.2)	0.64

VA: Vancomycin, COT: Cotrimoxazole, CIP: Ciproflaxicillin, DC: Doxycycline, AMX: Amoxicillin, ERYC: Erythromycin, AMP: Ampicillin, CRO: Ceftriaxone, CHL: Chloramphenicol, PEN: Penicillin, NB: Novobiocin, CLOXA: Cloxacillin, CET: Cefalotin, GEN: Gentamicin.

serving area.

The contamination level of AMR *E.coli* 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% (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 an MDR level of >70% (Mamza *et al.*, 2010). The difference might be due to rampant drug use (taking the medicines in a different way than the health care provider prescribed) in Ethiopia, bad hand hygiene of the FHs, food safety awareness of the FHs, and sanitation condition of the food serving area. The contamination rate of AMR *Salmonella* spp is also comparable to the study done in Bangladesh with a contamination rate of 30.25% and an 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% (Mama and Alemu, 2016; Wolde *et al.*, 2016). The difference might be due to the year of the study, personal hygiene of the FHs, food safety awareness of the FHs, and hygiene and sanitation condition of the food serving area.

The contamination rate of AMR *Shigella* spp is higher than the review conducted in Ethiopia with an overall contamination rate of 6.6% and MDR rate of 86.5% (Hussen *et al.*, 2019). However, a lower contamination rate and MDR level were observed in Pakistan (Saima *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 FHs, and hygiene and sanitation condition of the food preparation and serving areas.

The high contamination rate of FHs

with AMR enteric FBB is associated with short-time (5 years) work experience as a FHs, irrational drug use, lack of food safety training, educational status of the FHs, and poor sanitary condition of the food serving area

This is comparable to the study conducted in Dilla MCC, Ethiopia, where the hand of FHs contamination levels with AMR FBB *Salmonella* and *Shigella* spp was statistically associated with the educational status and service year of the FHs (Kuma *et al.*, 2020).

The main limitations of the study were: 1) the study done during the dry seasons but, the contamination of AMR enteric bacteria may vary throughout the seasons. 2) We used only 5 antibiotics for AMR testing because the study country Ethiopia commonly used those antibiotics only and available at the stock. 3) Study done at point in time (not longitudinal study) due to resources and time constraints.

Conclusions

AMR enteric *E. coli*, *Salmonella*, and *Shigella* spp were detected on food handlers at the DTU MCC. Due to this enteropathy, it could suggest the possibility of foodborne outbreaks and effect Food Security.

The contamination rate of FHs' hands with AMR FBB had a statistically significant correlation with the FHs' lack of work experience (less than 5 years), irrational drug usage, lack of food safety training, educational level, and the unsanitary conditions of FUs and food serving areas.

The finding implies that food safety training for FHs, the employment of experienced FHs, and close monitoring of the

implementation of approved hygienic standards could enhance irrational drug usage, sanitary conditions of FUs, and food safety in the MCC as a whole. In addition, reducing irrational medication usage could help minimize AMR in food and the environment.

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Table 3. Multiple logistic regression analysis that shows the association between the AMR enteric FBB contamination levels in the hands of the FHs of DTU with critical contributing factors.

Study variables		<i>E.coli</i>		<i>Salmonella</i>		<i>Shigella</i>		COR (95% CI)	AOR(95% CI)
		P	A	P	A	I	I		
Educational status	2ndary edu	53	11	37	27	21	43	1.53(1.35,1.74)	1.62(1.52,1.8)
	>2ndary edu	11	85	16	80	11	85	1	1
Work experience	< 2 years	54	5	32	27	16	123	1.31(1.01, 1.32)	1.42 (1.22, 1.87)
	2-5 years	11	85	21	75	16	80	1	1.02(0.99,1.5)
	>5 years	0	5	0	5	0	5	1	1
Food safety training	Yes	0	11	0	11	0	11	1.25(1.12,1.43)	1.52(1.32,1.6)
	No	64	85	53	96	32	117	1	1
Drug use characteristics	Rational	21	80	16	85	5	96	1	1
	Irrational	43	16	37	21	27	32	1.36(1.23,1.62)	1.75(1.64,2.0)
Sanitary condition of the food serving area	Clean	26	27	16	27	36	27	1	1
	not clean	84	23	94	23	74	23	1.47(1.30,1.59)	1.98(1.45,2.5)

P: Present, A: Absent, I: Reference.

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