ORIGINAL ARTICLE

Bacteriological Quality of Street Foods and Antimicrobial Resistance of Isolates in Hawassa, Ethiopia

Temesgen Eromo¹, Haimanot Tassew², Derese Daka³, Gebre Kibru²

ABSTRACT

BACKGROUND: Microbial contamination of ready-to-eat foods and beverages sold by street vendors and hawkers has become an important public health issue. In Ethiopia, health risks related to such kinds of foods are thought to be common. Thus, this study has tried to determine the bacteriological quality of ready- to- eat foods sold on streets.

METHODS: A cross-sectional study was conducted on street foods in Hawassa City from May to September 2014. A total of 72 samples from six food items such as local bread ('ambasha' and'kita'), raw fish, chilli ('awaze'), avocado and cooked potato were collected. Bacterial isolation, colony count and antimicrobial susceptibility testing were made following standard microbiological techniques.

RESULTS: About 31% of the food samples showed total colony counts ranging from $1.7x10^5$ to $6.7x10^6$ colony-forming unit per gram (CFU/g) which is beyond the acceptable limits set for microbiological quality of ready- to -eat foods. The mean coliform and Enterobacteriaceae counts in raw fish, 'kita' and 'ambasha' were also higher than the limits. E.coli was the most frequent isolate (29.6%) followed by Salmonella species (12.7% and S.aureus (9.9%). All isolates were 100% sensitive to ciprofloxacin. About 89% of Salmonella sp was resistant to chloramphenicol. Alarmingly, 14.3% of S.aureus was resistant to vancomycin.

CONCLUSION: This study confirmed considerable rate of contamination in street vended foods in Hawassa City. The identified foodborne bacteria and antibiotic resistance isolates could pose a public health problem in that locality. Therefore, regular inspection, health education and training of vendors on food handling and safety practices are recommended.

KEYWORDS: street foods, bacteriological quality, total aerobic count, Hawassa

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INTRODUCTION

Street foods are defined as ready-to-eat foods and beverages prepared and/or sold by vendors and hawkers, especially in streets and other similar public places (1). This industry plays an important role in meeting the food requirements of urban dwellers in many cities and towns of developing countries. It feeds millions of people daily with a wide variety of foods that are relatively cheap and easily accessible (2). However, food borne illnesses of microbial origin are a major health problem associated with street foods (3). Food

contamination with antibiotic resistant bacteria can also be a major threat to public health, since the antibiotic resistance determinants can be transferred to other pathogenic bacteria potentially comprising the treatment of sever bacterial infections (4.5).

The traditional processing methods that are used in preparation, inappropriate holding temperature and poor personal hygiene of food handlers are some of the main causes of contamination of street-vended foods.

Corresponding Author: Gebre Kibru, Email: gebre.tiga@ju.edu.et

¹Kembata Tembaro Zone Health Department, Durame, Ethiopia

²Department of Medical Laboratory Sciences and Pathology, Jimma University, Ethiopia

³Department of Medical Laboratory Sciences, Hawassa University, Ethiopia

Consumers who depend on such foods are more interested in its convenience and usually pay little attention to its safety, quality and hygiene (2,6).

Ready-to-eat street foods are also subjected to cross-contamination from various sources such as utensils, knives, raw foodstuffs, flies that sporadically landing on the foods, vendors bare hand serving and occasional food handling by consumers (6,7). In most cases, tap water is not available for washing hands and utensils at vending sites; hand and utensil washing are usually done in one or more buckets-sometimes without soap. Toilets, waste disposal and refrigeration facilities are rarely available. Wastewater and garbage are therefore discarded nearby, providing nutrients for insects and other household rodents, which may carry food borne pathogens (7).

Thus, potential health risks are associated with contamination of food by Escherichia coli, Salmonella typhi, Pseudomonas species, Staphylococcus aureus, Proteus species and other species during preparation, post-cooking and various handling stages (8, 9). As a result, food borne illness associated with the consumption of street foods has been reported in several places such as India (10), Mexico (11) and Ethiopia (12). In Ethiopia, health risks associated with street foods are common. Salmonella, Shigella and other food-borne pathogens were identified in similar studies on street-vended foods in this country (13,14).

There were reports of outbreaks that occurred due to consumption of street foods like raw/roasted fish and local bread in Hawassa, in the past two consecutive years (i.e. 2010 and 2011). The factors or causes associated with the occurrence of these outbreaks were not well identified (15). Additionally, there have been episodes of diarrhea related to consummation of ready-to-eat street foods among satellite cases (personal communications).

On the other hand, the prevalence of antimicrobial resistance among food borne pathogens has increased during recent decades (4, 5). A study in Benin revealed that 15.18% of *S. aureus* stains isolated from street foods were resistant to methicillin. All isolated bacterial colonies were resistant to Penicillin G. The bacterial colonies resistant to methicillin were also resistant to kanamycin, gentamycin, tobramycin

and erythromycin (16). Moreover, the Salmonella species isolated from street foods vended in Addis Ababa were sensitive to most of the drug tested. In a similar study, the isolated Shigella species showed multiple drug resistance patterns against trimethoprim-sulphamethoxazole, ampicillin, chloramphenicol, streptomycin and tetracycline (12). Since the popularity of street foods is increasing in the towns/cities in particular and in the country in general, this study was intended to the microbiological quality antimicrobial resistance status of bacterial isolates from ready- to- eat foods sold by street venders to ensure health status of the consumers.

MATERIALS AND METHODS

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Study design and setting: community based cross-sectional study was undertaken to determine the bacteriological quality of street foods in Hawassa City from May to September, 2014. The city had eight sub-cities and a total of 399,461 populations in 2014 (17). There are many resorts, hotels, restaurants and cafeterias in the city. Some of the common street foods vended in the city include: 'ambasha' (thin flat bread), 'kita' (pizza type bread), bombolino, cooked potato, potato chips, raw and roasted fish, 'awaze' (chili), boyina and slice of pineapples. Based on our assessment, the majority of the vendors were working at stationary (static) sites and none of them took formal training on food preparation and safety.

Regular street food vending areas in the city namely: nearby Lake Hawassa (Amora Gedel), around Bus station, Piazza, Kochi and old market place (Gebeya Dar) were selected purposively for sample collection. A total of 247 vendors were registered from those five places, and 72 participants were selected randomly from the list. For those vendors who retained two or more food items, only one food item was picked by lottery method. All street vended ready-to-eat foods with no further sort out ed, touched, uncovered or/and washed by customers were included in this study. Whereas any kind of fruits except processed avocado (salad form) and packed food types vended on the street were excluded because of having its own standards in interpreting microbiological load (18). In addition, fruits were excluded because of difficulty in tracing possible source of contamination.

Sample collection and processing: A total of 72 samples from different types of street foods (12 from each food item) such as local bread ('ambasha' and 'kita'), raw fish, chilli ('awaze'), avocado and cooked potato were collected and placed in labeled sterile plastic containers aseptically. Then, they were transported to the Referral Hawassa Hospital Microbiology Laboratory in cold box (block of ice in it) with 3-5°c and processed immediately as per the Laboratory Manual of Food Microbiology for Ethiopian Health and Nutrition Research Institute (EHNRI) (19).

A 10gm of food samples were homogenized in 90ml of buffered peptone water and shaken vigorously using vortex to dislodge adhered bacteria (19). The homogenate sample gave 1:10 dilution from which further dilution was made by adding 1ml of homogenate into 9ml of buffered peptone water. Depending on the level of contamination, serial dilutions of 10⁻² and up to 10⁻⁵ were also made for total aerobic count (TAC), coliform and *Enterobacteriaceae* counts (20). Then, the plate count was compared with the standard plate count level set for microbiological quality of ready-to-eat foods (18).

For total aerobic count, 1ml aliquots of appropriate dilution was poured on nutrient agar plates (Oxoid, Hampshire, UK)) and incubated at 30-35°c for 48±4 hours. Moreover, 1ml aliquots of appropriate dilution was poured on Eosin Methylene blue agar and incubated at 30-35°c for 24-48 hours, and dark blue-black colonies were counted as coliforms. For *Enterobacteriaceae* count, 1ml aliquots of appropriate dilution was pour edon MacConkey Agar plates and incubated at 30-35°c for 24-48 hours. Pink to reddish purple colonies with or without haloes of precipitate were considered as members of an *Enterobacteriaceae* family (20).

The remaining 1:10 diluted homogenate samples were inoculated on Manitol salt agar, Selenite F-broth, TCBS Agar and MacConkey agar plates and incubated at 37°C for 24 hours. Samples enriched by Selenite F-broth were again sub-cultured onto Xylose Lysine deoxycholate (XLD) agar. Then, the suspected colonies were sub-cultured into sterile nutrient broth for 2-4hrs, and from it, Gram-staining and a battery of biochemical tests (oxidase, catalase, simmon citrate, indole production, urease, motility,

coagulase, methyl red-Voges Proskaeur (MR-VP), lysine decarboxylase (LDC), Klingler's iron agar (KIA), mannitol fermentation, gas and H₂S production) were used to identify bacterial species (20, 21).

Antimicrobial susceptibility testing: Antimicrobial susceptibility test was carried out using Kirby-Bauer disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (22) on Mueller Hinton agar (Oxoid, Basingstoke, UK) with the following antibiotic discs: ampicillin (10 µg), erythromycin (15 μg), ceftriaxone (30 μg), cefotaxime (30 μg), chloramphenicol (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), cloxacillin (5 µg), oxacillin (1 µg), vancomycin (10 µg), trimethoprimsulfamethoxazole (1.25 µg), gentamicin (10 µg), clindamycin (2 ug), penicillin G (10 unit), norfloxacin (10 µg) and kanamycin (30 µg). These antimicrobial agents were selected based on the availability and frequency of prescription for the treatment of bacterial infections in Ethiopia. The reference strain Escherichia coli (ATCC 25923) was used as control to check the potency of antimicrobial disks.

Data analysis: Descriptive statistics was used to analyze the data. Frequency and mean were calculated for continuous variables. Then, findings were presented in the form of tables.

Ethical consideration: The study was ethically cleared by Jimma University, College of Health Sciences Ethical Review Board. Letter of support was also obtained from Hawassa City Health Department. Street food vendors were participated voluntarily after they were informed and signed the consent form. Any data regarding individual status of food handlers was kept confidential.

RESULTS

In this study, 31% of street vended foods showed high mean total colony count $(1.7 \times 10^5 \text{ to } 6.7 \times 10^6 \text{ CFU/g})$ compared to what is set for microbiological quality of ready-to-eat street foods. The mean aerobic counts of 'kita' $(6.1 \times 10^5 \text{ CFU/g})$, and 'ambasha' $(3.0 \times 10^5 \text{ CFU/g})$ (Table 1) were found to be beyond the acceptable level (below $10^5 \text{ CFU/g})$.

Similarly, the coliform counts of all tested food items (Table 1) were beyond the acceptable range. Relatively much higher coliform count was seen in raw fish than in other food types. 'Kita',

'ambasha' and raw fish also showed unacceptable level of *Enterobacteriaceae* count. However, the mean *Enterobacteriaceae* count obtained from

'awaze' (chili), and avocado (Table 1) was within the acceptable range.

Table1: Mean total aerobic bacterial, coliform and Enterobacteriaceae counts of ready to eat street foods against the standard Hawassa, Ethiopia, May to September 2014.

Food Item	Number	Mean bacterial colony	Coliform count	Enterobacteriaceae count				
		count (CFU/gm)	(CFU/gm)	CFU/gm)				
Kita*	12	6.1×10^5	7.6×10^3	$2.3x10^4$				
Ambasha**	12	3.0×10^5	2.6×10^3	1.1×10^4				
Raw fish	12	6.7×10^6	$5.1x10^4$	$6.8x10^4$				
Potato	12	4.0×10^5	$6.8x10^3$	$2.5x10^4$				
Awaze***	12	1.7×10^5	$3.9x10^3$	8.1×10^3				
Avocado	12	2.8×10^5	4.8×10^3	9.4×10^3				

^{*} Thin flat bread commonly made from Corn and Wheat flour and doesn't need any fermentation

The food samples taken from "Amora Gedl" (near Lake Hawassa) were more contaminated with bacteria compared to other street food samples sold at other sites (Table 2).

Table 2: Mean total aerobic bacterial, coliform and Enterobacteriaceae counts of ready to eat street foods with respect to Vending sites, Hawassa, Ethiopia, May to September 2014.

		Mean bacterial colony count (CFU/gm)										
Vending Place	Number -	Total aerobic count	Coliform count	Enterobacteriaceae count								
Bus Station	07	$3.8x10^4$	1.4×10^3	4.6×10^3								
Piazza	11	$6.3x10^4$	1.4×10^3	3.8×10^3								
Gebeya Dar	15	$1.7x10^6$	1.1×10^5	1.7×10^5								
Amora Gedel	28	3.5×10^6	1.5×10^5	4.4×10^5								
Kochi	11	$8.6 \text{x} 10^4$	1.4×10^3	2.8×10^3								

A total of 71 bacterial isolates which made up eleven genera were detected. Bacteria was more frequently detected in raw fish (24%, 17/71) followed by potato (18%, 13/71) and 'awaze' (14%, 10/71) (Table 3).

E.coli was the most frequent isolate (29.6%) followed by *Salmonella* and *Citobacter species* (12.7% each). However; *Edwardisella*, *M. morgan* and *Serratia* were present in low rate (1.4% each). The highest rate of *S. aureus* (3/7, 42.8%) was seen in 'awaze' while the highest rate of *E. coli* (5/21, 23.8%) and *Salmonella Spp.* (7/9, 78%) also observed in avocado and raw fish respectively (Table 3).

In this study, all isolates became 60-100% resistant to ampicillin. However, all of them were 100% sensitive to ciprofloxacin. S. aureus showed 100% resistance to cloxacillin, 28.6% to oxacillin 14.3% About 88.9% and vancomycin. Salmonella showed resistance sp to chloramphenicol 61.9% and E. to doxycycline. On the other hand, Salmonella, Proteus, Entrobacter, Klebisella and Citrobacter species showed no resistance to ceftriaxone, naldixic acid, norfloxacillin and trimethoprim sulphamethoxazole. Similarly, all isolates but E.coli, showed no resistance to gentamycin and kanamycin (Table 4).

^{**} Pizza type bread made from Wheat flour with baking powder

^{***}Chili-based thick sauce made from fresh red pepper, ginger, onions and salt and severely blended

Table 3: Bacterial isolates with respect to types of street foods, Hawassa, Ethiopia, May to September 2014.

Identified	Food Items												
organisms	Kita	Ambasha	Raw fish	Potato	Awaze	Avocado	Total Isolates n (%)*						
E. coli	2 (9.6)	4 (19)	4 (19)	3 (14.3)	3 (14.3)	5 (23.8)	21(29.6)						
S. aureus	0(0)	0(0)	0(0)	2(28.6)	3(42.8)	2(28.6)	7(9.9)						
Salmonella Spp	1(11)	0(0)	7(78)	0(0)	0(0)	1(11)	9(12.7)						
Proteus Spp	1(14.3)	2(28.5)	1(14.3)	1(14.3)	1(14.3)	1(14.3)	7(9.9)						
Klebisella Spp	0(0)	1(14.3)	0(0)	3(42.8)	1(14.3)	2(28.6)	7(9.9)						
Citrobacter Spp	3(33.3)	3(33.3)	2(22.3)	1(11.1)	0(0)	0(0)	9(12.7)						
Entrobacter Spp	2(40)	0(0)	2(40)	1(20)	0(0)	0(0)	5(7)						
Providenica Spp**	0(0)	0(0)	0(0)	1(33.3)	2(66.7)	0(0)	3(4.2)						
Others***	1(33.3)	0(0)	1(33.3)	1(33.3)	0(0)	0(0)	3(4.2)						
Total	10(14.1)	10(14.1)	17 (24)	13 (18.3)	10(14.1)	11(15.5)	71(100)						

^{*}Percentages were calculated by dividing the number of isolates in a particular food item by total number of isolates.

DISCUSSION

This study tried to examine the bacteriological quality of ready-to-eat street foods at selected five vending places in Hawassa, Ethiopia. Accordingly, 52%(37/71) of the food samples were found to be contaminated with food borne pathogens and indicator organisms including *E. coli, S. aureus* and *Salmonella sp.*

The total aerobic count (TAC) of 1.7×10^5 to 6.7×10^6 CFU/g obtained in those samples was also higher than 1.10 - 3.61×10^5 CFU/g reported in Gondar, Ethiopia (23), 2.98×10^3 CFU/g to 4.09×10^4 CFU/g in Lagos, Nigeria (24) and 12.16 - 25.81×10^5 CFU/g in Tirumala, India (25).

However, the mean TAC (5.4x10⁵ CFU/g) of this study was relatively lower than 1x10⁵ to 5.1 x10⁷ CFU/g reported in another study in Gondar (26), 10⁶ CFU/g in Hawassa (27) and, 10⁷ CFU/g in Addis Ababa (12). Such variations are largely attributed to differences in food contents, methods of preparation, personal hygiene, ways of handling and serving practices of vendors. The diversity in the environments/climatic conditions could be explanation for the additional observed differences. On the other hand, the mean TAC obtained in this study was comparable with findings in Kumasi, Ghana (28) whicht reported $5.9 \times 10^5 \text{ CFU/g}.$

^{**} Providencia Spp made of P. alkalifaciens and P. stuartii

^{****}Other represents *Edwardisella*, *M. morgani*, and *Serratia*; each organism had been isolated once

		Antibiotics tested															
Organisms	$N_{\underline{0}}(\%)$ of resistance																
	AMP	Cfx	CRO	CHL	CIP	CLI	CLX	DOX	E	GM	K	NA	NOR	OX	PG	SXT	VA
S. aureus	7		4	4	0 (0)	2	7	2	2	0 (0)			0 (0)	2	2	0 (0)	1
(n=7)	(100)		(57.1)	(57.1)		(28.6)	(100)	(28.6)	(28.6)					(28.6)	(28.6)		(14.3)
E. coli	21	2	3	7	0 (0)			13		2	2	2	4			6	-
(n=21)	(100)	(9.5)	(14.3)	(33.4)				(61.9)		(9.5)	(9.5)	(9.5)	(19)			(28.6)	
Salmonella	9		0 (0)	8	0 (0)							0 (0)	0 (0)			0 (0)	-
(n=9)	(100)			(88.9)													
Proteus	4 (80)	2(40)	0 (0)	0 (0)	0 (0)			1 (20)		0 (0)	0 (0)	0 (0)	0 (0)			0 (0)	-
(n=5) *																	
Klebisella	6 (86)	0 (0)	0 (0)	1 (14)	0 (0)			2(28.5)		0 (0)	0 (0)	0 (0)	0 (0)			0 (0)	-
(n=7)																	
Citrobacter	8 (89)	0 (0)	0 (0)	0 (0)	0 (0)			3(33.3)		0 (0)	0 (0)	0 (0)	0 (0)			0 (0)	-
(n=9)																	
Entrobacter	3 (60)	1(20)	0 (0)	0 (0)	0 (0)			1 (20)		0 (0)	0 (0)	0 (0)	0 (0)			0 (0)	-
(n=5)																	

Note: AMP, Ampicillin; CLI, Clindamycin; CLX, Clocxacillin; E, Erythromycin; OX, Oxacillin, PG, Penicillin G; VA, Vanvomycine; Cfx, Cefotaxime; CRO, Ceftriaxone; CHL, Chloramphenicole; CIP, Ciprofloxacin; GM, Gentamycin; K, Kanamycin; DOX, Doxycycline; NA, Naldixic acid; NOR, Norfloxacillin; SXT, Trimethoprim sulphamethoxazole. -- = not tested

Of the foods that showed unacceptably high mean aerobic counts were the two breads ('kita' and 'ambasha') which should have received some treatment unlike the raw fish, avocado and 'awaze'. This is due to the fact that those breads have frequent contacts with contaminated hands of venders during preparation, handling and serving.

In our study, the total coliform count ranged from 2.6×10^3 to 5.2×10^4 CFU/g which is much higher than 3×10^2 to 6.4×10^3 CFU/g reported in Gondar (23) and 2.8×10^2 to 3.99×10^3 CFU/g in Tirumala, India (25). However, this finding was to some extent comparable with 2.6×10^3 to 1.9×10^5 CFU/g documented in Addis Ababa (29). Similarly, the *Enterobacteriaceae* count of 8.2×10^3 to

6.8x10⁴ CFU/g seen in this study also by exceeded 1.0 to 4.7x10⁴ report made in Accra, Ghana (3) and 6x10 to 8x10² in Egypt (30). However, it was lower than 10⁵ CUF/g reported in Addis Ababa (13). Post-processing contamination and poor hygienic conditions of vendors and their vending vicinity could be possible reasons for the observed high coliform and *Enterobacteriaceae* counts in this study.

The food samples taken from "Amora Gedel" (near Lake Hawassa) were more contaminated with bacteria compared to foods from other street vending sites. The possible reason for this could be the vending environment which was filthy, filled with flies and wastes. In addition, the vendors often use lake water directly for washing utensils and raw fish.

We identified 30% of *E. coli* and 10% of *Salmonella* species which is incomparable with 72.5% *E. coli* and 57.5% *Salmonella* sp. documented in Bahirdar (31). The same type of bacterial isolates were also identified in macaroni and "bonbolino" in Gondar (23), street meat in Mekele, Ethiopia (32), red pepper, macaroni and other traditional foods in Ghana (28), rice, beans, fish, salad and other food items in Nigeria (24) and chili sauces in Mexico (11).

Enteropathogens are known to survive on the hands for three hours or longer (3) so that isolation of these organisms in this study is an indication of faecal contamination of the foods as it was suggested by Oluyege et al (33). This means, defective personal hygiene can facilitate the transmission of these pathogens via food to humans. In addition, Enterobacter sp., Serratia marcensens and Proteus sp. had been known to be implicated in many diseases including gastroenteritis (21). Therefore, the presence of members of family Enterobacteriaceae in food products is usually seen as health risk to microbial safety of the food.

The identification of salmonella species in raw fish would either come from contaminated waters or is contaminated by venders during preparation/handling. Therefore, proper sanitary measures need to be taken in serving ready-to-eat street raw fish.

The 10% S. aureus detected in the food samples examined in this study might be associated with improper personal hygiene and contaminated hands of vendors as this bacterium

usually is related to human skin and clothing. Even higher rate of *S. aureus* was reported in similar studies in Benin (16), Gondar, Ethiopia (23), Ghana (3) and Thailand (34) with rate of 56.2%, 51.8%, 39.1% and 17.9% in that order. As it was indicated by Mepba *et al* (35), the inherent danger in *S. aureus* with or without their metabolic products in various foods and without further heat treatment incurs possible outbreak of serious food borne illness. Moreover, the contamination of street foods with *S. aureus* which is attributed to non-adherence to standard hygienic practices during food preparation could lead to food poisoning.

Almost all tested enteric organisms except *E. coli* identified in this study were 100% sensitive to ciprofloxacin, gentamycin, naldixic acid, norfloxacillin and trimethoprim sulphamethoxazole. This means that these antimicrobials are still drug of choice for the management of food borne illnesses in that locality.

On the other hand, S. aureus evolved resistance to ampicillin, cloxacillin, ceftriaxone and other tested antimicrobial drugs which would make the treatment of S. aureus infections difficult. About 28.6% of S. aureus isolates were also resistant to oxacillin which is a bit higher than 15% resistance reported in Benin (16). However, it is much lower than 90% resistance to the same drug seen in Jimma, Ethiopia (36). At this point, it is important to remark that bacterial isolates resistant to oxacillin eventually become resistant to chloramphenicol, cloxacillin, doxycycline, erythromycin and penicillin G which may make the treatment of S. aureus infection more difficult. The 28.6% Oxacillin resistant S.aureus isolates were supposed to be resistant to methicillin too. As explained by Zhang et al. (37), the emergence of methicilin resistant S. aureus (MRSA) strains has become a major concern in medical practice. Alarmingly, in our study, about 14.3% of S. aureus isolates were resistant to vancomycin which is currently the main antimicrobial agent available to treat life threatening infections with MRSA.

Salmonella sp. showed 100% resistance to ampicillin and 88.9% to chloramphenicol indicating that those drugs will no longer be used for treatment of salmonella infection. However, it was 100% sensitive to ciprofloxacin and 89% to

naldixic acid which is in agreement with study findings in Bahir Dar, Ethiopia where 100% sensitive to the former and 78% to the later drugs was reported (31).

In general, this study demonstrated that street vended foods which are sold on the streets of Hawassa were considerably contaminated. The foodborne bacteria and antibiotic resistance isolates detected in this study are also evident that street foods might pose a major problem for public health. Lack of training (orientation) on the proper handling and processing of food, poor personal hygiene of venders and unhygienic surroundings could be possible factors for observed problems in that locality. Therefore, education for vendors on food safety and hygienic practices is essential to reduce contamination rate. In addition, regular inspection on food vending practices and safety of street foods is required to improve the health standards of consumers.

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