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PARASITOLOGICAL SURVEY AND ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF MICROBIAL ISOLATES FROM PRE WASHED FRUITS AND VEGETABLES COLLECTED IN METTU TOWN, I/A/BORA ZONE, SOUTHWEST ETHIOPIA

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Abstract:

Background*: Intestinal parasitic infections are widely distributed throughout the world causing substantial intimidation to the public health, economy, and physical and cognitive development particularly among children in developing countries like Ethiopia. Fruits and Vegetables may be contaminated at any point during growing, harvesting, sorting, packaging, and storage. This study was aimed at assessing parasitological survey and antimicrobial susceptibility patterns of microbial isolates from fruits and vegetables collected in Mettu town, Ethiopia from March – October, 2018.****Methods****:A cross sectional study design was conducted. A total of 240 fresh fruits and vegetable samples were collected on different days from local markets. Four types of fresh vegetable comprising lettuce, carrot, tomato, green pepper and two type fruits comprising lemon and banana were collected from selected local markets. For microbiological analyses, 25g of sample was aseptically weighed and washed gently in 225ml of sterile 0.1% (w/v) peptone water (Oxoid) for 3 minutes. Bacterial isolation and antimicrobial susceptibility was performed. For parasitological survey a portion fruit and vegetable was washed separately in 500 mL of normal saline for detaching the parasitic stages (ova, larvae, cysts) of helminthes and protozoan parasites commonly assumed to be associated with vegetable contamination. After overnight sedimentation of the washing solution, 15 mL of the sediment was then transferred to a centrifuge tube using sieve, to remove undesirable matters. For concentrating the parasitic stages, the tube was centrifuged at 3000 rpm for five minutes. After centrifugation, the supernatant was decanted carefully without shaking. Then the sediment was agitated gently by hand for redistributing the parasitic stages. Finally, the sediment was examined under a light microscope using ×10 and ×40 objectives. **Results:** A total of 240 samples of fruits and vegetables were collected from the local markets and examined for microbial isolate and parasite contamination .A total of 196 bacterial isolates of eight genera were identified. Klebsiella spp. 40(20.4%) was the most dominant followed by Citrobacter spp. 38(19.4%), Enterobacter spp. 32(16.3%), E. coli 24(12.2%), Salmonella spp.22 (11.3%), Proteus spp.20 (10.2%), S. aureus 20(10.2%), More than 90% of microbial isolates were resistant for Ampicillin and amoxicillin. All the S. aureus isolates were sensitive to oxacillin and vancomycin. Nearly 93.8% of the isolates were sensitive for ciprofloxacillin. whereas 41.5 % of the isolates showed resistance to oxytetracycline.. Resistance to nitrofurantoin, nalidic acid, streptomycin, chloramphenicol, cotrimoxazole, ceftriaxone, kanamycin and Gentamycin were 30.7%,28.4%,19.9%,17%,11.4%,14.8%,10.2%, 6.8% respectively. The results of parasitological examination showed that 100 samples were identified to be microscopically positive with at least one type of parasite, which gave rise to the overall contamination rate of 41.7%. The stages and species of parasites detected include ova of Ascaris lumbricoides(32%), Hymenolepis nana(16%), and cysts of Giardia lamblia(28%), and Entamoeba histolytica/dispar(24%).*

Conclusion*: Fruits and vegetables in Mettu local markets were significantly contaminated with potentially pathogenic bacteria, multiple antibiotics resistant and the potential source of transmission for intestinal parasites to humans. Prevention of contamination remains the most effective way of reducing food borne parasitic infection. A comprehensive health education should be given to vendors and farmers of fruits and vegetables and to the general population on the health risks associated with consumption of contaminated fruits and vegetables. Further studies should be conducted on the viability of parasitic contaminants of fruits and vegetables. Also, other researches must be done to evaluate the level of parasitic contamination of farm produces, water, and soil.*

Key words: ***Parasitological survey, vegetable, microbial isolate, antibiotic resistance , Mettu***

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INTRODUCTION:

Background

Globally, food borne illnesses are accountable for significant morbidity and mortality. Factors contributing to the emergence of foodborne diseases are changes in human demographics and behavior, technology and industry, and international travel and commerce; microbial adaptation; economic development and land use; and the breakdown of public health measures(1–3)

Intestinal parasitic infections are widely distributed throughout the world causing substantial intimidation to the public health, economy, and physical and cognitive development particularly among children in developing countries like Ethiopia. The poor personal hygiene, poor environmental hygiene, and poor health system commonly observed in developing countries make the prevalence to be highest among these populations (4,5). The consumption of fruits and vegetables helps in protecting human body from a number of diseases by providing nutrients, vitamins, minerals, protein, and fibers(6,7). It could also have a positive impact on body-weight regulation and related conditions, including diabetes and hypertension. However, fruits and vegetables, especially, those that are consumed raw and or not properly washed, have been the major way for the transmission of human pathogens(8–10).

Intestinal parasitic infection may be acquired in different ways like by consumption of contaminated fruits, vegetables, other food stuff, and water(11–14). Eating unclean, raw, or undercooked fruits and vegetables is one of the means by which the transmission of intestinal parasitic infections is propagated(15). Fruits and vegetables act as vehicles for the transmission of parasitic infections when contaminated as a result of various associated factors related to planting, such as while they are still on the field, harvesting, transportation, storage, market chain, and even at home (16,16–19).

Outbreaks of illness caused by bacteria, viruses and parasites have been linked epidemiologically to the consumption of a wide range of vegetables. Surveillance of vegetables has indicated that these foods can be contaminated with various bacterial pathogens, including *Salmonella*, *Shigella*, *E. coli O157:H7*,*Listeria monocytogenes* and *Campylobacter*. However, the prevalence of foodborne pathogens on vegetables and their involvement in outbreaks are not well documented in developing countrie(20–23).

The emergence of antimicrobial resistance in disease-causing bacteria is a public health concern that poses unique communication challenges(21,21,24,25). Antimicrobials are essential for treating infectious disease in both humans and animals. However, their improper use may lead to the emergence of new strains of bacteria that cannot be treated with commonly used antimicrobials. Sometimes, pathogens emerge that are resistant to multiple antimicrobials, making treatment extremely difficult. A further complication is that antimicrobials are commonly used in food animals, such as cattle, swine, and poultry, which are a common source of exposure to human pathogens linked to food(26).

The prevalence of antimicrobial resistance among food borne pathogens is reported to have increased, probably as a result of selection pressure created by the use of antimicrobials in agriculture and animal health(26–28)

Most dangerous microorganisms do not change the appearance of the food, so we usually can't tell that the food is contaminated with dangerous microor-ganisms by just looking, smelling or tasting it. Several surveys have demonstrated the presence of pathogenic enteric bacteria on produce and in unpasteurized fruit or vegetable juices and vegetable sampled during production or at retail markets in different parts of the world among them some survey

were done in Jimma(29,30), Nekemte(31),Mekele(32), Kenya(33),Sudan(34), Nigeria(35,36)

To our researcher's knowledge, there is no published document to attest the level of parasitic contamination and antimicrobial susceptibility patterns of microbial isolates of fruits and vegetables in Mettu Town. Therefore, this study was designed to determine the level of parasitic contamination and antimicrobial susceptibility patterns of microbial isolates from pre washed fruits and vegetables collected in selected local market of Mettu town, southwest, Ethiopia.

METHODS:

Study site. The study area was Mettu town, the capital town of Ilu Aba Bora Zone, which is located 600 km away from Addis Ababa, in the southwestern part of the country. It covers 16884 square kilometer and has an estimated population of 1361582. There are three hospitals, a number of health centers.

Study design and period. A cross-sectional study was conducted from March to October 2018 in Mettu town, southwest Ethiopia.

Sample Collection and Analysis. Fruits and vegetables including lemon, banana, lettuce, carrot, tomato, green pepper brought to Mettu town for sale during the study period was the target sample for this study. A purposive sampling technique was employed to collect a total of 240 fruits and vegetable samples. The entire sample was collected and put in sterile plastic containers, properly labeled and transported within 3hrs of sample collection to the microbiology and parasitology laboratory of biomedical Department, Mettu University and also to Mettu karl referral hospital laboratory for microbiological and parasitological analysis. Microbiological analysis was conducted within three hours of sample collection.

Data collection

For parasitological survey a portion fruit and vegetable was washed separately in 500 mL of normal saline for detaching the parasitic stages (ova, larvae, cysts) of helminthes and protozoan parasites commonly assumed to be associated with vegetable contamination. After overnight sedimentation of the washing solution, 15 mL of the sediment was then transferred to a centrifuge tube using sieve, to remove undesirable matters. For concentrating the parasitic stages, the tube was centrifuged at 3000 rpm for five minutes. After centrifugation, the supernatant was decanted carefully without shaking. Then the sediment was agitated gently by hand for redistributing the parasitic stages. Finally, the sediment was examined under a light microscope using ×10 and ×40 objectives.

Microbiological analysiss: For microbiological analyses, 25g of sample was aseptically removed from each sample using a sterile spatula and gently shaken in 225ml of sterile 0.1% (w/v) bacteriological peptone water (Oxoid) for 3 minutes. Serial dilutions of 10^{-2}, $_{10}{}^{-3}$ were made and then 0.1ml of the suspension from each dilutions were plated in duplicate on a pre-dried surfaces of nutrient agar and average count were recorded after multiplying by reciprocal of dilution factor and reported as colony forming unit per gram.

For isolation of *Salmonella*spp and *Shigella* spp. Vegetable samples (25 g) was added to 225 ml bacteriological peptone water, gently shaken and the suspension incubated at 37ºC for 24 hours for the metabolic recovery and proliferation of cells. From this, 1ml of culture was transferred into tubes containing 10 ml of Selenite F Broth. Selenite F broth was incubated at 37ºC for 24 hours. After enrichment, culture from selenite F broth was separately streaked on plates of MacConkey Agar, Xylose Lysine Desoxycholate (XLD) medium (all from Oxoid). Characteristic colonies that are non-lactose fermenterswith black centers from each selective medium was picked, purified and tested biochemically on Kligler‟s Iron Agar (Oxoid), Lysine Iron Agar (LIA) (Oxoid), Urea Agar (Oxoid), oxidase, indole, Simmons Citrate Agar (Oxoid) and SIM Medium (Oxoid). For isolation and identification of *S. aureus*, a loop full of sample from the homogenate was inoculated on Manitol Salt Agar (MSA) and yellow colonies on MSA which was catalase positive and coagulase positive isolates was identified as *S. aureus*.

Drug susceptibility testing: The criteria used to select the antimicrobial agents tested was based on the availability and frequency of prescription for the management of bacterial infections in Ethiopia and WHO Recommended antimicrobials for surveillance of *Salmonella* and *E. coli* as they are the major food borne pathogen around the globe.

Antimicrobial susceptibility testing for microbial isolate was performed using the disk diffusion method and results were interpreted using the criteria of the CLSI (Clinical and laboratory standard institute). The antibiotics used were Ampicillin (10µg) ,Amoxicillin (10µg),Chloramphenicol(30µg)

,Streptomycin (10 µg) ,Oxytetracycline (30µg), Cotrimoxazole (1.25µg) ,Ciprofloxacillin (5µg) ,Gentamycin (10µg), Nalidic acid (30µg) Kanamycin (30µg), Ceftriaxone (30µg). Antibiotic susceptibility testing for *S. aureus* was determined for Erythromycin (E-15 µg), Oxacillin (Ox -1 µg), in addition to the above antibiotics. A standard reference strains *E. coli* (ATCC 25922) and S.aureus (ATCC 25923) with known sensitivity were used in this study. Interpretation of readings as sensitive, intermediate or resistant was made according to a standard chart

Data Quality Control

The following measures was undertaken so as to control the quality of the data and laboratory investigation. Properly designed and pre-tested data collection instrument was used. Every day the collected data was cross checked for completeness, consistency and on site corrective action was made.

A standard operational procedure tools was strictly used for sample collection, transportation, processing and storage. Special emphasis was given during coding each culture media as well as the collected vegetable samples. Before use all disks, reagents and culture media was checked being at appropriate temperature and within specified shelf life. Antibiotic sensitivity test was performed according to clinical laboratory standard institute guide. *E. coli* strain ATCC 25922 and *S. aureus* strain ATCC 25923 was included. For plasmid isolation, optimization test were performed for gel concentration, running time and volt.

All media were checked visually before being inoculated for any change in appearance that could indicate contamination or deterioration.

Microbial isolates

Statistical Analysis

Data were organized and summarized in simple descriptive statistics methods. Moreover, all components of the data entered and analyzed using SPSS 20.0 computer software. Chi-square test (X^2) results were used and a *p*-value of less than 0.05 was considered statistically significant.

Ethical Consideration

Ethical clearance was obtained from Mettu University research and ethical review committee. The purpose and procedures of the study was explained to the respondents (vendors of vegetables) a verbal consent was obtained from all study participants. Privacy and confidentiality of the study participants response and laboratory test result was maintained. Results

Parasitological Survey

A total of 240(40 sample for each)fresh fruits and vegetable samples were collected on different days from local markets. Four types of fresh vegetable comprising lettuce, carrot, tomato, green pepper and two type fruits comprising lemon and banana were collected from selected local markets and examined for microbial isolate and parasitological contamination. The results of the study showed that 100 samples were identified to be contaminated with at least one type of parasite, which gave rise to the overall contamination rate of 41.7%. The stages and species of parasites detected ova of Ascaris lumbricoides (32%), Hymenolepis nana(16%), and cysts of Giardia lamblia(28%), and Entamoeba histolytica/dispar(24%).

Table 1: Prevalence of bacteria isolated from fresh fruits and vegetables collected in selected local market of Mettu town southwest Ethiopia, 2018

Microbial isolates	Frequency(n)	Percent (%)
Klebsiella spp	40	20.4
Citrobacter spp	38	19.4
Enterobacter spp	32	16.3
E.coli	24	12.2
Salmonella spp	22	11.3
Proteus spp	20	10.2
S.aureus	20	10.2
Total	196	100.0

A total of 196 bacterial isolates of eight genera were identified. Klebsiella spp. 40(20.4%) was the most dominant followed by Citrobacter spp. 38(19.4%), Enterobacter spp. 32(16.3%), E. coli 24(12.2%), Salmonella spp.22 (11.3%), Proteus spp.20 (10.2%), S. aureus 20(10.2%) (**table-1**).

The presence *E.coli* in freshly consumed vegetables was indicators of poor sanitation condition and presence of potential pathogenic microorganisms such as salmonella. Moreover, detection of *Salmonella spp* in 25 gm of fruits and vegetable samples is considered as unacceptable for consumption. The presence of *S.aureus* in fresh fruits and vegetable samples indicates direct hand contact by venders of fruits vegetables as these organisms are present on skin of as normal flora (**table-1**).

Antimicrobial susceptibility

A total of 196 bacterial isolates of eight genera were identified. Klebsiella spp. 40(20.4%) was the most dominant followed by Citrobacter spp. 38(19.4%), Enterobacter spp. 32(16.3%), E. coli 24(12.2%), Salmonella spp.22 (11.3%), Proteus spp.20 (10.2%), S. aureus 20(10.2%), More than 90% of microbial isolates were resistant for Ampicillin and amoxicillin. All the S. aureus isolates were sensitive to oxacillin and vancomycin. Nearly 93.8% of the isolates were sensitive for ciprofloxacillin. whereas 41.5 % of the isolates showed resistance to oxytetracycline.. Resistance to nitrofurantoin, nalidic acid, streptomycin, chloramphenicol, cotrimoxazole, ceftriaxone, kanamycin and Gentamycin were 30.7%,28.4%,19.9%,17%,11.4%,14.8%,10.2%, 6.8% respectively(**table-1&2**).

Table 2Antibiotic resistance patterns of gram negative rods isolated from fresh fruits and vegetables sold in local market of Mettu town southwest Ethiopia, 2018

Bacterial isolate		Antimicrobial agents											
	Numb	**AM**	**AMX**	**C**	**S**	**SXT**	**OXT**	**CIP**	**GN**	**NA**	**K**	**CRO**	**F**
Klebsiella spp	40 % 	40 100	36 90	4 10	7 17.5	-	8 20	- 27	3 7.5	10 25	3 9	5 36	9 22.5
Citrobacter spp	38 %	37 97	36 94.7	- 16.6	4 10.5	4 10.5	10 26.3	3 7.9	-	9 23.7	3 7.9	3 7.9	11 28.9
Enterobacter spp	32 %	30 93.8	31 96.9	7 21.9	5 15.6	3 9.4	8 25	-	5 15.6	4 12.5	8 25	5 15.6	7 21.9
E.coli	24 %	15 62.5	15 62.5	9 37.5	9 37.5	- 10.5	12 50	7 29	-	8 33	3 12.5	8 33	12 50
Salmonella spp	22 %	22 100	22 100	9 40.9	-	1 4.5	18 81.8	- 4.8	1 4.5	5 22.7	1 4.5	5 22.7	5 22.7
Proteus spp	20 %	20 100	20 100	1 5	10 50	12 60	17 85	1 5	3 15	14 70	-	-	10 50
Total	176 % 	164 93	160 90	30 17	35 19.9	20 11.4	73 41.5	11 6.2	12 6.8	50 28.4	18 10.2	26 14.8	54 30.7

Key: AM=Ampicillin (10µg), AMX=Amoxicillin (10µg) ,C=Chloramphenicol(30µg) ,S=Streptomycin (10 µg), SXT= Cotrimoxazole (1.25µg) ,OXT=Oxytetracycline(30µg) ,CIP=Ciprofloxacillin (5µg) GN=Gentamycin (10µg) ,NA=Nalidic acid (30µg) ,K=Kanamycin (30µg),CRO=Ceftriaxone (30µg) F=Nitrofurantoin (300µg)

Table 3: Antibiotic resistance patterns of S.aureus isolatedfrom fresh fruits and vegetables sold in selected local market of Mettu town southwest Ethiopia, 2018

Bacterial isolate		Antimicrobial agents														
	Numb	AM	AMX	C	S	SXT	OXT	CIP	GN	NA	K	CRO	F	OX	VA	E
S.aureus	20 %	-	13 65	-	8 40	-	-	-	-	2 30	-	-	-	-	-	-

Key: AM=Ampicillin (10µg), AMX=Amoxicillin (10µg) ,C=Chloramphenicol(30µg) ,S=Streptomycin (10 µg), SXT= Cotrimoxazole (1.25µg) ,OXT=Oxytetracycline(30µg) ,CIP=Ciprofloxacillin (5µg) GN=Gentamycin (10µg) ,NA=Nalidic acid (30µg) ,K=Kanamycin (30µg),CRO=Ceftriaxone (30µg) F=Nitrofurantoin (300µg), OX=Oxacillin (1µg) ,VA=Vancomycin (30µg) ,E=Erythromycin (15µg)

DISCUSSION:

A total of 240 samples of fruits and vegetables were collected from the local markets and examined for microbial isolate and parasitological contamination. The results of the study showed that 100 samples were identified to be contaminated with at least one type of parasite, which gave rise to the overall contamination rate of 41.7%. The stages and species of parasites detected ova of Ascaris lumbricoides(32%), Hymenolepis nana(16%), and cysts of Giardia lamblia(28%), and Entamoeba histolytica/dispar(24%).

The present study demonstrated heavy microbial contamination of fresh vegetables sold in the open markets with the ranges of total aerobic mesophilic counts between 10^6-10^7 CFU/g for all vegetable samples. Studies elsewhere have investigated the microbiological quality of street vended foods in different countries; high bacteria counts and a high incidence of food borne pathogens in such foods have been reported. The microbial load of vegetables in the current study area is higher compared to study done in Accra(40)for tomato sample and comparable with study done in Nigeria(37–39) Accra(40)but

lower compared to study done in Ghana, documented in the street foods of Kumasi (41). The discrepancy between the present study and previous studies might be as a result of the variations in geographical locations, seasonal, climatic and environmental conditions, the kind of sample and sample size examined, the sampling techniques, methods used for detection of the microbial isolates and socioeconomic status.

More than 90% of vegetable samples had total viable counts of greater than 10^6CFU/g. Similar study conducted in Addis Ababa reported over 90% of the vegetable samples had aerobic mesophilic counts of ≥ log 6 CFU/g(40). The high microbial load of vegetable in this study could be due to fact that open markets vegetables were seen displayed on open stalls in close proximity to waste container without lids where flies are swarming all over the place, mostly close to open gutter, direct hand contact during both harvest and sell.

In the present study a total of 196 bacterial isolates identified. A total of 240 samples of fruits and vegetables were collected from the local markets and examined for microbial isolate and parasite contamination .A total of 196 bacterial isolates of eight genera were identified. Klebsiella spp. 40(20.4%) was the most dominant followed by Citrobacter spp. 38(19.4%), Enterobacter spp. 32(16.3%), E. coli 24(12.2%), Salmonella spp.22 (11.3%), Proteus spp.20 (10.2%), S. aureus 20(10.2%), More than 90% of microbial isolates were resistant for Ampicillin and amoxicillin. All the S. aureus isolates were sensitive to oxacillin and vancomycin. Nearly 93.8% of the isolates were sensitive for ciprofloxacillin. whereas 41.5 % of the isolates showed resistance to oxytetracycline.. Resistance to nitrofurantoin, nalidic acid, streptomycin, chloramphenicol, cotrimoxazole, ceftriaxone, kanamycin and Gentamycin were 30.7%,28.4%,19.9%,17%,11.4%,14.8%,10.2%, 6.8% respectively.

The prevalence of *E.coli* in the present study was lower compared to study done in Lebanon(42) which reported prevalence of (42.30%) this could be due to sample type, sample size, climatic and seasonal variation. However the present study was comparable with study done in Lebanon(42) in demonstrating the presence of pathogenic microorganism in fresh vegetables consumed which are usually consumed raw and represent a risk for human health(30,37,43–46).

The presence of antibiotic-resistant bacteria in fresh vegetables may constitute food safety concern since bacteria serving as a reservoir for resistance determinants may have great influence on resistance gene transfer in natural habitats, such as the human colon, fruit and vegetable surface[4, 28, 29]. More than 90% of microbial isolates were resistant for ampicillin and amoxicillin. All the *S. aureus* isolates were sensitive to oxacillin and Vancomycin.

Antibacterial resistance is a worldwide threat and concerns have arisen about the involvement of commensal and pathogenic bacteria in the maintenance and spread of resistance genes.Results of the present study demonstrated multiple antibiotic resistance isolates from fresh fruits and vegetable samples which was comparable to a report from Nigeria(37) Addis Ababa(47), Jimma(30).

The detection of intestinal parasitic stages from fruits and vegetables is an indicative of the fecal contamination from human and or animal origin. As in many tropical countries, intestinal parasites are widely distributed in Ethiopia not only due to the favorable climatic conditions for the survival and dissemination of the parasites but also due to the unsanitary conditions that facilitate fecal pollution of water, food stuffs, and soil(48).

The present study has attempted to assess the level of contamination and prevalence of different intestinal parasites from different fruits and vegetables sold in selected markets of Mettu Town. The overall parasitic contamination rate was found to be 41.7%, which is in agreement with the findings reported elsewhere (31,49,49,50). However, it is higher than what was reported in similar studies from other areas(32,51–53). On the other hand, it is lower when compared with the findings of some studies(54–56).
The discrepancy between the present study and previous studies might be as a result of the variations in geographical locations, climatic and environmental conditions, the kind of sample and sample size examined, the sampling techniques, methods used for detection of the intestinal parasites, and socioeconomic status. So long as these factors differ, consequently the discrepancy of the results would be expected.

No ova of hookworm species were detected from the samples examined in the present study. This is in agreement with other studies conducted in Jimma town(29). This might be due to the fact that hookworms have very short life span in the soil(57).

CONCLUSION:

Fruits and vegetables in Mettu local markets were significantly contaminated with potentially pathogenic bacteria and multiple antibiotics resistant and the potential source of transmission for intestinal parasites to humans. Prevention of contamination remains the most effective way of reducing food borne parasitic infection. A comprehensive health education should be given to vendors and farmers of fruits and vegetables and to the general population on the health risks associated with consumption of contaminated fruits and vegetables. Further studies should be conducted on the viability of parasitic contaminants of fruits and vegetables. Also, other researches must be done to evaluate the level of parasitic contamination of farm produces, water, and soil.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

All the authors conceived the study, participated in the study design, data collection, data analysis, and drafted the paper for publication. All authors have read and approved the final paper.

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