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Identification, Antimicrobial Resistance Pattern and Community Knowledge, Attitude and Practices of *Salmonella* in Mizan Town, Ethiopia: Cross Sectional Study

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Abstract

Salmonella is the leading cause of foodborne diseases worldwide. A cross-sectional study was conducted between the periods of March and October 2019 at municipal abattoir and butcher houses of Mizan town, Ethiopia with the objectives to determine the prevalence, antimicrobial resistance pattern, risk factors and assess public awareness of Salmonella. A total of 320 samples consisting of 240 from abattoir and 80 from butcher houses were collected and examined for the presence of Salmonella using the procedures outlined by the International Organization for Standardization. The overall prevalence of Salmonella was found to be 13.4% (43/320). Out of a total isolates, 30/240 (12.5%) were isolated from abattoir source, of which 21/175 (12%) from carcass swab, 4/25 (16%) from abattoir personnel hand swab and 5/40 (12.5%) from abattoir materials swab while 13.3/80 (16.2%) from butcher houses source, of which 5/30 (16.6%) from butcher personnel hand swab and 8/50 (16%) from butcher materials swab. However, there was no statistically significant difference (P>0.05) in the prevalence of salmonella among sample source and type. Out of the total 43 isolates, 42(97.67%) were multiple antimicrobial resistant and the highest level of resistance was observed for ertrymycin (100). Multivariable logistic regression result showed that, materials which were not cleaned and people who didn't know contamination as risk were the major risk factors for the occurrence of Salmonella among abattoir and butcher houses in the study area.Besides, the knowledge, attitude and practices of beef meat handlers were founded to be poor. Thus, urgent intervention program is essential to minimize the risk associated with consumption of beef meat contaminated with Salmonella and prudent use of antimicrobialsis recommended.

Keywords: Abattoir; Antimicrobial; Butcher; Beef meat; Prevalence; Salmonella; Mizan

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Introduction

Salmonella is considered the most prevalent foodborne pathogen worldwide and has long been recognized as an important zoonotic microorganism of economic significance in animals and humans. Consumption of raw or unsafe food, cross-contamination, improper food storage, poor personal hygiene practices, inadequate cooling and reheating of food items, and a prolonged time lapse between preparing and consuming food items were mentioned as contributing factors to an outbreak of salmonellosis in humans [1]. The ubiquity of *Salmonella* isolates creates a persistent contamination hazard in all raw foods and also in animal-origin food products, which are often implicated in sporadic cases and outbreaks of human salmonellosis [2].

Besides, antibiotic-resistant *Salmonella* infections of both human and animal is a concern, particularly in developing countries where the risk of infection is high because of unhygienic living conditions, close contact and sharing of houses between animals and humans [2,3].

In the study area there is much less information on the knowledge, attitudes and practices (KAP) around meat safety; the gender and social determinants of meat safety; or the relation between hazards in meat and health outcomes in consumers of meat in the country. In line with the aforementioned limitations in the study area the prevalence of *Salmonella* species andtheirantimicrobial

resistance patternas well as the knowledge, attitude and practice of the community was not yet known. Therefore, this study was designed with the objectives to determine the prevalence and antimicrobial resistance pattern of *Salmonella* isolates from slaughtered cattle, personnel and materials in the abattoir and butcher houses, to observe risk factors associated for the occurrence of *salmonella*and to assess the knowledge, attitude and practice of meat value chain: abattoir workers and butchers on meat hygiene and safety.

Materials and Methods

Study period and area

The study was conducted between the periods of March and December 2019 in Bench Maji zone, at Mizan municipal abattoir and Butcher houses.

Study population and sampling

The study populations were all apparently healthy local indigenous zebu cattle which were brought to the abattoir for slaughtering. We were using systematic random sampling procedure to select our study animals to take swab sample from carcasses. Samples were also taken from cattle meat handlers (abattoir workers, butchers) and materials in contact with meat.

Sample size determination

For isolation and prevalence of *salmonella* from carcass, sample size was calculated according to Thrusfield[4] using 95% confidence level and 5% precision. The 12.5% expected prevalence [5] of *salmonella* from carcass in agro ecologically similar study area, in Southwest, Ethiopia was used.

For questionnaire survey, observation, personnel hand swab and material samples, the sample size was determined purposively based on the willingness of the interviewees, ease for follow up, the total number of persons engaged and the availability of materials to be sampled in the abattoir and butcher houses. Accordingly, 145 samples were taken.

Study design

A cross-sectional study involving microbiological analysis, questionnaire survey and observational survey was employed.

Sampling technique and sample collection

A total of 320 samples consisting of 240 from Abattoir and 80 from Butcher houses were sampled. Systematic random sampling technique was used for carcass swabs and purposive sampling technique was used for personnel and materials swabs. Swabs from carcass were taken from the abdomen (flank), thorax (lateral), crutch, and breast (lateral) while both the right and left hands were swabbed for personnel hand swabs and all surfaces of the materials were swabbed thoroughly. All samples were labeled legibly with permanent marker identifying type/ source of sample and date of sampling. Finally, by using ice boxes with ice packs the samples were transported to Mizan Regional Veterinary Laboratory, South West Ethiopia.

Isolation and identification of *salmonella*

Isolation and Identification of *Salmonella* organisms were carried out according to [6,7]. Accordingly, Non-selective pre-enrichment, Selective enrichment, Plating on selective media and Biochemical confirmation were used.

Pre-enrichment

The swabs were directly inoculated into 10 ml buffered peptone water (BPW) in screw capped bottles and incubated at 37°C for 16-18 hrs. Each 25 ml of the swab content was inoculated into 225 ml of BPW and homogenized for two minutes with stomacher. After mixing thoroughly, the samples were incubated at 37°C for 16-18 hours [6].

Selective enrichment

From the pre-enrichment broth after incubation and thoroughly shaking, 0.1 ml of the broth was transferred into a tube containing 10 ml of Rappaport-Vassiliadis medium (RV broth). Then 1 ml of the pre-enrichment broth was transferred into a tube containing 10 ml of Selenite broth (SB broth). The inoculated RV broth was incubated at 41.5°C ± 1°C for 24 ± 3 hours and the inoculated SB broth at 37°C ± 1°C for 24 ± 3 hours [6].

Plating and identification

Xylose lysine desoxycholate (XLD) agar plate was used for plating and identification purpose. A loop-full of inoculum each from the RV and SB broth was transferred and streaked separately onto the surface of XLD agar. The plates were incubated at 37°C ± 1°C for 24 ± 3 hours. The plates were examined for the presence of suspected *Salmonella* colonies, which on XLD agar were pink with a darker center and a lightly transparent zone of reddish color due to the color change of the indicator whereas lactose positive *salmonellae* were yellow with or without blackening. Presumptive colonies were transferred to nonselective solid media for further confirmatory tests. Confirmation was done by using biochemical test according to [6].

Biochemical Tests

Triple sugar iron agar

A loopful culture of pure growth from nutrient agar was stabbed into the butt and streaked on the slant and incubated for 24 hours at 37°C. Typical *Salmonella* cultures showed alkaline (red) slants and acid (yellow) butts with gas production (bubbles) and formation of hydrogen sulfide (blackening of the agar) [6].

Urea agar

The isolates were inoculated into the urea to determine urease production. The inoculated tubes were incubated at 37°C for up to 96 hours. Then an observation was made at an interval of 4, 24, 48 and 96 hours. Urease positive cultures changed the color of the indicator to red.

Citrate utilization test

The colonies were cultured on the prepared Simmon's citrate agar medium, incubated at 37°C for 48 hours and observations

were recorded. Opacity and change in color of bromothymol from green to blue indicated a positive reaction.

Lysine decarboxylation medium

Lysine decarboxylation broth was inoculated with the loopful culture of the test organism and one was kept uninoculated control. Both tubes were incubated for 24 hours at 37°C. Turbidity and a purple color after incubation indicated a positive reaction. A yellow color indicated a negative reaction.

Indole test

Peptone water was prepared and the ingredients were dissolved in distilled water, dispensed in test tubes and sterilized by autoclaving at 121°C for 15 minutes. The tubes of the medium were inoculated with test isolates using sterile platinum loop and incubated at 37°C aerobically for up to 96 hours. Finally, 0.5 ml of Kovac's reagent was added to each of the inoculated and un-inoculated controls. The tubes were shaken gently and the results were recorded. Positive results were indicated by the development of red colour in the alcoholic layer of the reagent and no colour in the control tube.

Antimicrobial resistance pattern tests

The antimicrobial resistances testing of the isolates were performed by using the disc-diffusion method according to the recommendations of the National Committee for Clinical Laboratory Standards [8]. Four to five well-isolated colonies from nutrient agar plates were transferred into tubes containing 5 ml of Tryptone soya broth (Oxoid, England). The broth culture was incubated at 37ºC for 4 hours until it achieved the 0.5 McFarland turbidity standards. Sterile cotton swab was dipped into the suspension, rotated several times, pressing firmly on the inside wall of the tube above the level to remove excess inoculums and swabbed uniformly over the surface of Muller Hinton agar plate (Oxiod, England). The plates were held at room temperature for 30 min to allow drying.

The resistance of the isolates were tested for the following antibiotic discs: Ampicillin (AMP) 2 µg, Oxicillin (OX) 5 µg, Gentamicin (HLG) 120 µg, Kanamycin (K) 5 µg, Ox tetracycline (O) 30 µg, Erythromycin (E) 5 µg, Neomycin (N) 30 µg and Penicillin G (P) 1 µg were placed at least 15 mm apart from the edge of the plates to prevent overlapping of the inhibition zones. The plates were incubated at 37ºC for 24 h. The diameter of the zones of inhibitions was compared with recorded diameters of the control organism E. coli ATCC 25922 and classified as resistant, intermediate, or susceptible according to the interpretive standards of the Clinical Laboratory Standards Institute [9].

Statistical Analysis

Descriptive statistics such as frequency, percentage, and/or proportion were used for prevalence, antimicrobial resistance test, questionnaire survey and observation results. Chi-squire test was used to assess significant differences of *Salmonella* status between sample source and types while Binary Logistic regression (odds ratio) was used to assess the association of possible risk factors for the occurrence of *Salmonella*using statistical package for social science (SPSS) version 20 software. The results with less than P-value of 0.05 were considered statistically significant.

Ethical consideration

The study was approved by the Research Ethics Committee and the letter of clearance was obtained from Jimma University College of Agriculture and veterinary Medicine, and Bench Maji zone administration office. The data was collected after written informed consent was made with all study participants. All the rights of privacy and confidentiality of participants were protected.

Results

Over all occurrence of *Salmonella*

The overall prevalence of *Salmonella* in this study was found to be 13.4% (43/320) with prevalence of 12.5% in abattoir and 16.2% in butcher houses. Statistical analysis of the data showed that there was no statistically significant difference (P>0.05) on the prevalence of *Salmonella* between abattoir and butcher houses sources **(Table 1).**

Occurrence of *salmonella* isolates among sample types

The specific prevalence of *salmonella* was found to be 12% in carcass swab, 16% in abattoir personnel hand swab, 12.5% in abattoir materials swab, 16.6% in butcher men hand swab and 16% in butcher materials swab.-The lowest prevalence was observed from carcasses samples among the others. The prevalence of *Salmonella* retrieval was not statistically significant (P>0.05) among the sample types **(Table 2).**

Antimicrobial resistance pattern test

Out of the total 43 isolates subjected to antimicrobial resistance test to 8 different antimicrobials, the highest level of resistance was observed for erythromycin (100%) followed by ampicillin (83.7%), oxacillin (72.09%) and neomycin (67.44%). All isolates were found to be susceptible to gentamycin **(Table 3).**

Out of the total isolates, 42/43 (97.67%) were resistance to at least one antimicrobial agents tested **(Table 4).**

Occurrence of *salmonella* among risk factors

Out of 145 purposive samples expected to be potential risk factors (abattoir worker=25, abattoir materials=40, butchers=30 and butcher house materials=50), a total of 22 (15.1%) *Salmonella* was isolated. The specific prevalence of *Salmonella* was found to be 16% (4/25), 12.5% (5/40), 16.6% (5/30) and 16% (8/50) respectively in abattoir workers, abattoir materials, butchers and butcher house materials.

Table 1 Proportion of *Salmonella* isolates from Abattoir and Butcher Houses.

Source of samples	Number Examined	Prevalence (%)	χ^2	P-value
Abattoir	240	30 (12.5)	0.725	0.449
Butcher house	80	13 (16.2)		
Total	320	43 (13.40		

Table 2 Prevalence and Association of *Salmonella* Recovery between Sample Types.

Sample type	Total Observation	Frequency of Positivity	Prevalence	χ^2	P-value
Carcass swab	175	21	12%	1.033	0.905
Abattoir personnel hand swab	25	4	16%		
Abattoir materials swab	40	5	12.5%		
Butcher men hand swab	30	5	16.66%		
Butcher materials swab	50	8	16%		

Table 3 Antimicrobial Resistance Pattern of *Salmonella* from Abattoir and Butcher Houses.

Antimicrobials	Disc concentration (µg)	Number of isolates		
		Resistant (%)	Intermediate (%)	Susceptible (%)
Ampicillin (AMP)	2	36(83.7)	7(16.2)	-
Oxacillin (OX)	5	31(72.09)	-	12(27.9)
Gentamicin (HLG)	120	-	-	43(100)
Kanamycin (K)	5	22(51.16)	14(32.55)	7(16.27)
Oxy tetracycline (O)	30	12(27.9)	-	31(72.09)
Erythromycin (E)	5	43(100)	-	-
Neomycin (N)	30	29(67.44)	14(32.55)	-
Penicillin G (P)	1	19(44.18)	17(39.53)	7(16.27)

Table 4 Multiple Antimicrobial Resistance Patterns of *Salmonella*.

No. Isolates with same pattern	Antimicrobial resistance pattern	No. of antimicrobials developed resistance
12	ERY	1
10	AMP, OX	2
8	OXY, PEN, KAN	3
6	ERY, AMP, KAN, PEN	4
4	KAN, AMP, PEN, GEN, N	5
3	N, ERY, PEN, KAN, AMP, OXY	6

OXY: Ox tetracycline; ERY: Erythromycin; KAN: Kanamycin; AMP: Ampicillin; OX: Oxicillin; PEN: Penicillin; N: Neomycin

Table 5 Univariable and Multivariable Logistic Regression analysis of the association of risk factors for the occurrence of *salmonella* among Abattoir and Butcher houses.

Risk factors	Categories	Frequency	Positive No. (%)	Univariable OR (95% CI)	*P-value*	Multivariable OR (95% CI)	*P-value*
Educational status	Illiterate	24	9(37.5)	4.2(0.9-18.5)	0.046	7.12(0.31-163)	0.219
	1-8	74	6(8.1)	0.52(0.12-2.2)	0.385	1.19(0.095-15)	0.891
	9-12	29	4(13.7)	0.17(0.01-1.8)	0.150	0.16(0.001-21)	0.468
	> grade 12	18	3(16.6)	**		**	
Hand washing	before& after	28	2(7.1)	**		**	
	Before	70	7(10)	2.5(0.29-22.0)	0.400	0.96(0.06-15.6)	0.980
	After	13	4(30.7)	2.2(0.13-39.0)	0.578	0.63(0.05-77.0)	0.851
	not wash	34	9(26.4)	18.9(2.29-155)	0.006	3.43(0.20-57.0)	0.390
Manner of cleaning equipment	water&detergent	63	2(3.1)	**		**	
	water only	23	5(21.7)	4.09(0.99-16.8)	0.051	18.8(0.80-441)	0.068
	not wash	59	15(25)	4.16(1.27-13.6)	0.018	12.5(0.98-160)	0.048
Manner of hand washing	water & detergent	78	1(1.28)	**		**	
	water only	34	7(20.5)	0.36(0.04-3.14)	0.358	0.18(0.06-6.0)	0.341
	not wash	33	14(42)	10.0(3.40-29.4)	0.000	5.4(0.73-40.78)	0.097
Job related training	No	119	13(10)	0.39(0.14-1.08)	0.072	0.33(0.03-3.2)	0.346
	Yes	26	9(34.6)	**		**	
Job related medical test	No	128	16(12)	0.10(0.033-0.3)	0.000	0.11(0.01-1.03)	0.054
	Yes	17	6(35.2)	**		**	
Using protective clothes	No	80	15(18)	0.24(0.09-0.6)	0.007	0.74(0.09-5.8)	0.778
	Yes	65	7(10.7)	**		**	
Cleaning equipment	No	59	13(22)	3.03(1.18-7.7)	0.021	2.5(0.30-21.3)	0.386
	Yes	86	9(10.4)	**		**	
Using detergents	No	101	12(11)	0.36(0.14-0.9)	0.034	0.09(0.01-1.0)	0.050
	Yes	44	10(22)	**		**	
Personal hygiene	No	91	10(10)	0.83(0.33-2.1)	0.699	0.80(0.08-7.2)	0.847
	Yes	54	12(22)	**		**	
Know contamination as risk	No	33	14(42)	22.7(7.36-70.1)	0.000	11.5(1.6-80.9)	0.014
	Yes	112	8(7.1)	**		**	

CI=Confidence interval; OR=odd ratio; **=Reference point

Table 6 The Knowledge, Attitude and Practice of Abattoir Workers.

Factors	Values	Frequency	Percentage (%)
Educational status	Illiterate	14	23.23
	1-8	25	41.66
	9-12	14	23.23
	beyond grade 12	7	11.66
Placement in the abattoir	Slaughteringa	25	41.66
	Loading	16	26.66
	Washing stomach	11	18.33
	Washing the intestine	8	16.66
Job related training	Yes	12	20
	No	48	80
Job related medical test	Yes	6	10
	No	54	90
Know contamination as risk	Yes	39	65
	No	21	35
Clean clothing	Yes	22	36.66
	No	38	63.33
Hand washing	Before & after	4	6.66
	Before	11	18.33
	After	29	48.33
	Not wash	16	26.66
Knives are clean	Yes	38	63.33
	No	22	36.66
Unhygienic equipment placing	Yes	37	61.66
	No	23	38.33

a=Cutting the throat, flaying eviscerating, splitting the carcass and carcass washing

The association of *Salmonella* recovery in personnel and materials with the possible risk factors by Univariable logistic regression reveled that; those personnel who were not educated (Illiterates) have 4.23 times more likely the chance of contaminating carcass than the other categories of educational status (95% CL: 0.966-18.528:p=0.046), people who did not wash their hands during meat processing have 18.9 times more likely the chance of contaminating meat with *Salmonella* comparing with those who wash their hands at least before or after contact with meat/equipment (95% CL: 2.292-155.82: p=0.006).

With regarding to cleaning equipment, those materials which have not been cleaned regularly have 3 times more likely the chance of contaminating meat than equipment that regularly washed (95% CL: 1.181-7.788 with p=0.021).While abattoir workers and butchers who did not knew contamination as risk have 22.7 times more likely the chance of cross contaminating carcasses in comparison to those who knew contamination as risk (95% CL: 7.367-70.180 with p=0.000) and also workers who used jewelry materials on their hands during meat processing have chance of 4.3 times more likely to contaminate meat comparing with those who did not used (95% CI 1.680-11.250 with p=0.002).

Job related training and personal hygiene were not significantly associated with the occurrence of *salmonella* (p>0.05; table 5). All significantly associated variables (p<0.25) in univariable logistic regression analysis were taken to multivariable logistic regression analysis to control confounders.

In multivariable logistic regression analysis the occurrence of *salmonella* isolates in abattoir and butcher houses were more likely higher in materials which were not cleaned (OR=12.56; 95% CI: 0.986-160.13%; *P*=0.048) and people who didn't know contamination as risk (OR=11.586; 95% CI: 1.65-80.98%; *P*=0.014) than other manners of cleaning equipment categories and in those who know contamination as risk respectively **(Table 5).**

Questionnaire and observational survey

For questionnaire survey analysis, a total of 145respondents used, of which 60 from Abattoir workers and 85 from Butchers houses. Twenty two (36.66%) of the workers use unclean knives while 37 (61.66%) of them keep equipment in unhygienic places.Whilst 43 of the respondents responded that unclean hand and equipment as major causes of carcass contamination, sixteen considered falling on the ground as a major source of contamination. Washing the hands before and after work is practiced by only four of the interviewees and thirty eight did not regularly put on clean protective clothing at work. Only seven of them responded that the faeces, skin and dirty water could possibly cause carcass contamination. Most (65%) interviewees consider that keeping hygiene is the role of the management while some (35%) of them think the role of management is setting standards for hygiene in abattoir and workers role is maintaining standards for hygiene in the slaughterhouse.

Direct observations revealed the absence of hot water, sterilizer, carcass retention room and all processes were achieved in a single floor of the abattoir. During slaughtering equipments were placed on unclean surfaces. Knives were placed on the floor, in

Table 7 The knowledge, attitude and practice of Butcher house workers.

Factors	Values	Frequency	Percentage (%)
Educational status	Illiterate	10	11.76
	Grade 1-8	49	57.64
	Grade 9-12	15	17.64
	Beyond grade 12	11	12.94
Received job related training	Yes	14	16.47
	No	71	83.52
Received job related medical test	Yes	11	12.94
	No	74	87.05
Apron(protective clothes)	Used	44	51.76
	Not used	41	48.23
Jewellery materials	Worn	20	23.52
	Not worn	65	76.47
Hand washing	Before and after	9	10.58
	Before	17	20
	After	41	48.23
	Not wash	18	21.17
Manner of hand washing	Using detergent and water	23	27.05
	Rinsing with water only	44	51.76
	Not wash	18	21.17
Handling money	Cashier	23	27.05
	Butcher with bare hand	62	72.94
Cleaning equipment at the end of work using water & saop	Yes	48	56.47
	No	37	43.52
Use detergents	Yes	26	30.58
	No	59	69.41
Cutting table	Single	57	67.07
	Separate for d/t organs &meat	28	32.94

their (workers) mouth, on the skin of killed and in the anus of a slaughtered animals. The protective clothes were unclean, blood tinged and frequently in contact with carcasses (**Table 6**).

Among the 85 butchers, 71 acquired meat selling skills from observations and fourteen of them from informal training. Forty one of the butchers did not use protective clothes and forty four of them wash their hands with only water after work. All reported that they use a single knife for cutting meat and edible offal. Twenty had worn jewelries and sixty two handled money while selling meat. Forty eight of the butchers cleaned their shop and equipment every day at end of the selling process by using water and soap (**Table 7**).

Discussions

In the present study, the overall prevalence of *Salmonella* was 13.4% (43/320). This finding agrees with previous studies undertaken in different parts of Ethiopia which was 14.8% at Dessie[10] and 12.5% at Wolaitasodo[5].

Resistance to multiple antimicrobials (97.67%) which was observed in current study was in line with the reports of Asrat, [11] who revealed 95.45%.

The occurrence of *Salmonella* in the study area was directly or indirectly associated with the risk factors since *Salmonella* is cross contaminant of foods mainly meat. The current finding is in agreement with the studies conducted in Ethiopia, which showed that people and equipments were found to be significantly associated with carcass contamination by *Salmonella*[10].

The majority 37(61.66 %) of the abattoir workers proposed unclean hand and equipment as the major causes of carcass contamination but few responded that the faces, skin and dirty water can cause carcass contamination. Besides, most consider that keeping hygiene is the role of the management while some of them think the role of management is setting standards for hygiene in abattoir and workers role is maintaining standards for hygiene in the slaughterhouse.

The hygienic practices at the butcheries were unhygienic. Most of the butchers (72.9%) handle money with bare hands while processing meat and do not put appropriate protective clothes. Similarly, Molla [12] and Yismaw et al [13] reported 91.7% and 95% of the butchers in handle money while processing meat. In addition, other study indicates that, handling of foods with bare hands may also result in cross contamination, hence introduction of microbes on safe food.

Conclusion

This study revealed that high prevalence of *Salmonella*, presence of poor personal hygiene, resistance of *Salmonella* to most antimicrobials, low level of public awareness about contamination of beef meat with *Salmonella* and the associated risk factors for

the occurrence of *Salmonella* in the study area. Consequently, beef meat provided to the consumers in the town was found to be poor quality and risk full for human health calling for urgent intervention.

Authors' Contributions

AA: Study conduction, data collection, analysis, reference search, and manuscript writing; MA: Study conduction, reference search, manuscript writing, and editing. HD: analysis, reference search, and manuscript writing. All authors have read and approved the final manuscript.

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Computing Interests

The authors declare that they have no competing interest.

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