Detection and Determination of *Staphylococcus aureus* in Camel Milk and Associated Factors in Fedis, Eastern Hararghe, Ethiopia

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ABSTRACT

BACKGROUND: Camel milk is the key food for pastoralists in the arid and semi-arid areas of the eastern lowlands of Ethiopia. Unless it is handled under good hygienic conditions, milk can be a good medium for bacterial growth and can lead to foodborne diseases caused by various pathogenic microorganisms, such as *Staphylococcus aureus*. The current study was aimed to determine the prevalence of *Staphylococcus aureus* in raw camel milk and associated factors in Fedis, Eastern Ethiopia.

METHODS: A cross-sectional study was conducted from December 2, 2018 to May 26, 2019 in Fedis, Eastern Hararghe, Ethiopia. The questionnaire was used to determine the hygienic practices of camel milkers and sellers. Laboratory analysis was conducted to determine the level of *Staphylococcus aureus*. A total of 156 (78 from households and 78 from selling sites) milk samples were collected randomly from selected camel herd owners and selling sites for the isolation and enumeration of *Staphylococcus aureus*. Data analysis was carried out using SPSS software version 22.0. Finally, Chi-square and Fisher's exact tests were used to analyze the data. A *P*-value of .05 was considered as the cut-off point for statistical significance.

RESULTS: A total of 156 samples, including 78 samples of raw milk from the households and 78 samples from the market were purchased to determine *Staphylococcus aureus*. Out of 156 samples, *Staphylococcus aureus* was detected in 60 (38.5%) samples, of which 27 (34.6%) and 33 (42.3%) were among those collected from household and market, respectively. The overall mean *Staphylococcus aureus* count was 4.83 log CFU/mL, with household and market samples accounted for 2.76 and 5.08 log CFU/mL, respectively. Furthermore, 23 (38.3%) of the samples were contaminated with *Staphylococcus aureus* beyond the recommended level (4-5 log CFU/mL). There was a statistically significant association (*P*-value of $<.05$) between the prevalence of *Staphylococcus aureus* and other variables such as age of the camel, parity, lactation stages, and sources of milk.

CONCLUSION: More than one-third of milk samples were contaminated with *Staphylococcus aureus* at levels higher than the maximum permitted level. The findings of the current study suggests that there is a potential risk of foodborne infection and intoxication. Therefore, implementation of adequate hygiene and safety practices is very important to prevent the consumption of contaminated fruit juices, which leads to foodborne illness.

KEYWORDS: *Staphylococcus aureus*, microbiological quality, milk, bacteriological quality, food safety

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Introduction

Milk is a major source of food, nutrition security, and household income and also serves as a significant cultural function for the pastoral communities in Eastern African countries like Somalia, Sudan, Ethiopia, and Kenya.[1] In most pastoralists' areas, camel milk is always consumed either fresh or with varying degrees of sourness in its raw state without treatment or adequate preservation, which can pose a health hazard to the consumer.[2] Camel milk is a major source of food, nutrition security, and household income and also serves as a significant cultural function for the pastoral communities in Eastern African countries, including Somalia, Sudan, Ethiopia, and Kenya.[1]

Currently, foodborne pathogen is considered as one of the world's leading causes of disease outbreaks related to consumption of contaminated food.[3] Globally, foodborne illnesses are responsible for an estimated 600 million cases and 420 000 deaths.[4] Unless it handled, processed and stored under the hygienic condition, milk is a good medium for the growth of various pathogenic microorganisms, including *Staphylococcus aureus* (*S. aureus*). Because, milk is highly perishable, it supports

the growth and multiplication of pathogenic organisms leading to food spoilage, foodborne infection and poisoning.[5]

S. aureus is one of the most important cause of food-borne illnesses in the world due to its capability to produce a wide range of heat-stable enterotoxins.[6,7] *S. aureus* is an important pathogen in raw milk and the main pathogens involved in mastitis worldwide.[8-10] It is one of the leading sources of intramammary infections.[11,12] *S. aureus* is among the major cause of morbidity and significant economic losses. Similarly, it is responsible for causing clinical and sub-clinical mastitis.[6,7] *S. aureus* is an important pathogen that can cause *Staphylococcal* Food Poisoning (SFP).[13] For example, in United States, about 240 000 cases of SFP occur each year leading to hospitalization in 1000 cases and death of 6 people.[14]

However, food borne diseases, including SFP are more prevalent in developing countries and there is no reliable estimates.[15,16] Consumption of raw milk and its products such as cheese, cream, and yogurt is common in sub-Saharan Africa, including Ethiopia.[16,17] However, the production and consumption of raw milk and various dairy products often takes place under poor hygiene conditions.[16,18] However, still there is a limited evidence on the prevalence of *S. aureus* in camel milk and determinant factors in Ethiopia, which cover both production and market areas.

Therefore, the current study was aimed to provide the evidences on the prevalence of *S. aureus* in camel milk and factors associated with contamination of milk in Fedis, Eastern Hararghe, Ethiopia from December 2, 2018 to May 26, 2019. The findings of this study can be used by health program planners, policy makers, and other concerned bodies to develop appropriate intervention strategies and to improve the quality of milk and to protect consumer health.

Materials and Methods

Study area, design, and period

A cross-sectional study was conducted from December 2, 2018 to May 26, 2019 in Fadis, Eastern Hararghe, Ethiopia. It is located 539 km east of Addis Ababa at a geographical coordinate of 8° 49′ 43.3″N latitude and 42° 0′ 45.57″E longitude and an elevation of 1285 m. The district consists of 19 rural kebeles (the smallest administrative unit) and 2 urban kebeles. The livelihood of the population is 93.8% agro-pastoralist while the rest, 6.2% are urban dwellers.

Sample size determination

The sample size was calculated by using the single population proportion formula:

$$n = \frac{1.96^2 * \text{Pexp}(1 - \text{Pexp})}{d^2}$$

where n is the minimum sample size required, p is the estimated proportion of prevalence, and Zα/2 is the value of the standard score at 95% confidence interval. The assumptions of confidence level are at 95% = 1.96, a margin of error (d) = 0.05. For this study, p = 11.45% (previous prevalence) was used.[19]

Finally, a total of 156 milk samples were included in the study, of which 78 milk samples from households and 78 raw milk samples from market or selling sites were collected for the isolation and enumeration of *S. aureus*. Similarly, each 50 camel owners who are involved in camel milk production and handling were randomly selected and interviewed at different sites (3 selected kebeles, Kufa-Bobasa, Riski, and Agudora).

Sampling procedures and techniques

Multi-stage cluster sampling was used in the current study. The Peasant Association (PA) was considered the primary unit, and camel herd owners were secondary units. The district and PAs were chosen based on the camel population, camel milk production and marketing, pastoralist willingness, and vehicle accessibility. Then, 3 PAs, namely Kufa-Bobasa, Riski, and Agudora, were purposively selected. The camel owners who have lactating camels were asked to register for participation. Using this registration as a sampling frame, the herds were selected using simple random sampling.

Data collection and methods for face-face interview

The respondents were interviewed using a semi-structured questionnaire to assess the hygienic practices of camel milk handling. This data collection tool includes the questions used to collect data regarding: udder, buckets, and hand washing practice; sources of water supply; milking of camels with mastitis; milking frequency; milking order; milking time; condition of milk storage; storage equipment hygiene; awareness of public health importance and other conditions that may affect the hygienic quality of raw milk.

Sample collection and processing

The simple random sampling method was used to collect the milk samples from selected households and selling sites. Two hundred fifty milliliters of milk samples were collected aseptically from the udder and selling equipment in sterile test tubes. Aseptic technique was used throughout sampling and handling procedures by using sterile materials, flaming, and refrigeration. Sterile containers, including sampling materials were used for sample collection using the aseptic technique. Each sample was labeled and placed in a cool box with ice until transported to the veterinary microbiology laboratory at Haramaya University. The samples were transported to the laboratory using the ice-box and kept at 4°C and immediately analyzed within 2 hours. The samples were subjected to microbiological analysis within 24 hours. Similarly, information such as the source of milk, the condition of the milk, the time of sale, and the distance from the milk sources to the market were recorded during the collection.

Laboratory analysis

Isolation and enumeration of Staphylococcus aureus. All media used for bacteriological analysis were prepared according to the manufacturer's recommendations. Serial dilutions of 6-folds (10^{-1} to 10^{-6}) were done based on ISO 6887-1:1999 protocols.[20] Similarly, triplicate, 3 plates for each dilution were taken from each set of serial dilution (10^{-1}, 10^{-2}, and 10^{-6}) to determine the *S. aureus* in milk samples. A 1 mL of milk samples was diluted in 9 mL of Buffered Peptone Water (BPW) in a flask to make 10–1 dilution. A 1 mL from the first dilution (10^{-1}) was transferred into a second test tube containing 9 mL of BPW to get a 10^{-2} dilution. The same procedure was used to get the serial dilutions of 10^{-3} to 10^{-6}. For each step, the dilutions were mixed using a vortex mixer for 5 to 10 seconds, and it is used for the isolation and enumeration of *S. aureus*.

1 mL of the milk sample was inoculated on sterile Mannitol Salt Agar (MSA) plates at each dilution (10^{-1} to 10^{-6}). These diluted samples were then spread on the media surface by using sterile swabs, and the plates were allowed to dry for about 15 minutes. Then the plate was kept inverted and incubated at 37°C for 24 hours to allow bacterial growth. After 24 hours, characteristic appearances of yellow colonies on MSA were considered presumptive *S. aureus* colonies. Then, the colonies were converted into colony forming units per milliliter (CFU/mL) using the formula[21]:-

$$N = \frac{\sum C}{V(n1 + 0.1n2)d}$$

- ΣC = the sum of the colonies identified on all plates.
- V = the volume of inoculum on each plate in milliliters.
- n_1 = the number of plates selected at the first dilution.
- n_2 = the number of dishes selected at the second dilution.
- d = the dilution rate corresponding to the first dilution.

The total number of microorganisms enumerated per mL of the sample was calculated using the number of colonies obtained from each plate. Finally, the results were presented as the number of *S. aureus* per mL, and reported as log CFU/mL.

Gram's stain and biochemical tests. All suspected cultures of *S. aureus* were subjected to Gram's stain and observed under a light microscope for gram's reaction, size, shape, and cell arrangements. The gram-stained smears from typical colonies that showed gram-positive cocci occurring in bunched, grape-like, irregular clusters were taken as presumptive *S. aureus* colonies.[22]

For biochemical test, the pure culture colonies were taken from MSA plates, streaked on Nutrient Agar Plate (NAP), and incubated for 24 h at 37°C. The pure isolates in the nutrient slant will be preserved and maintained at *+40°C* for characterization of the isolates by biochemical tests. The pure culture of the isolates was picked up by bacteriological loop from the NA plate and mixed with a drop of 3% H_2O_2 (FACT health materials) on a clean slide for the catalase test. For catalase test, a pure culture of the isolates were picked using a sterile loop from the agar slant and mixed with a drop of 3% H_2O_2 on a clean glass slide. If the organism was positive, bubbles of oxygen were liberated within a few seconds. The catalase positive cocci were considered as *S. aureus*.

After inoculation of 0.1 mL of the test sample on sterile MSA plates from each dilution, the presence of growth and a change in pH in the medium (red to yellow) was considered as a presumptive identification of *S. aureus*. The coagulase tests (both slide and tube coagulase tests) were used in the current study. The culture colonies of *S. aureus* were picked from NAP by bacteriological loop, and placed on a clean slide with a small drop of distilled water and emulsified. The test suspension was treated with a drop of rabbit plasma and mixed well with a needle for 5 to 10 seconds. Those forming clusters of cocci were taken as coagulase positive *S. aureus*[22].

The tube coagulase test was performed by adding 0.5 mL of selected isolates of *Staphylococcus* grown on Tryptone soya broth (TSB) at 37°C for 24 hours to 0.5 mL of citrated rabbit plasma. Then, the tubes were incubated at 37°C along with a negative control tube containing a mixture of 0.5 mL of sterile TSB and 0.5 mL of rabbit plasma. Clotting was evaluated at 30 minutes intervals for the first 4 hours of the test and then after 24 hours incubation period. It was considered positive, if any degree of clotting from a loose clot to a solid clot that is immovable when the tube is inverted.[23]

Data processing and analysis

All data obtained through laboratory analysis and a questionnaire survey was entered into a Microsoft Excel spread sheet. Data analysis was carried out using SPSS software version 22.0. The microbial counts were first transformed into colony forming units per milliliter of sample (log CFU/mL) and the results were presented as the geometric means and other descriptive statistics. The association between the prevalence of *S. aureus* in milk samples and other variables was determined using the Chi-Square tests and Fisher's exact tests. Finally, a *P*-value of $<.05$ was considered as a cut point for statistical significance.

Data quality control

The questionnaire and observation checklist were pretested on 5% of the study sample size, outside the study areas, using face-to-face interviews to ensure clarity and applicability of the questionnaire. To minimize cross contamination, standard microbiological procedures were strictly followed while conducting the experiments. The aseptic technique was used for sampling, handling and testing.[24]

Table 1. Socio-demographic characteristics of the respondents in Fedis, Eastern Ethiopia.

VARIABLES	CATEGORIES	CAMEL MILKER'S (N = 50) (%)			MILK SELLERS (%)	TOTAL (%)
		K/BOBASA (N = 17)	RISKI (N = 16)	AGUDORA (N = 17)	BOKO (N = 50)	
Sex	Male	9 (52.9)	11 (68.8)	7 (41.2)	27 (54)	74 (54)
	Female	8 (47.1)	5 (31.2)	10 (58.8)	23 (46)	26 (46)
Age	Young	7 (41.2)	7 (43.8)	6 (35.3)	26 (52)	51 (51)
	Adults	10 (58.8)	9 (56.2)	11 (64.7)	24 (48)	49 (49)
Education level	Primary level	4 (23.5)	3 (18.8)	7 (41.2)	18 (36)	32 (32)
	Illiterate	13 (76.5)	13 (81.2)	10 (58.8)	32 (64)	68 (68)

Table 2. Prevalence of *Staphylococcus aureus* in raw camel milk samples collected from Fedis district, Eastern Ethiopia.

SAMPLE TYPES	EXAMINED SAMPLES	POSITIVE SAMPLES (%)	MEAN (LOG CFU/ML)	CATEGORY	
				1-3 LOG CFU/ML	4-5 LOG CFU/ML
Household milk	78	27 (34.6)	2.76	17 (62.96)	10 (37.04)
Market milk	78	33 (42.3)	5.08	20 (60.6)	13 (39.4)
Total	156	60 (38.5)	4.83	37 (61.7%)	23 (38.3%)

Abbreviation: CFU, colony forming unit.

While conducting the experiments throughout the study, the techniques were kept consistent to reduce errors, using sterile materials, flames, and refrigeration. Positive control tubes with citrated plasma and coagulase producing strain of *S. aureus* ATCC 25923 were used in the current study. Autoclave was used to sterilize the medium and equipment at 121°C for 15 minutes. Furthermore, 70% ethyl alcohol was used for disinfection.[24]

Results

Socio demographic characteristics

One hundred respondents who had camels or camel milk sellers were interviewed using a pre-structured questionnaire to assess the hygienic practices of camel milk handling from primary production to final selling site. Among total participants, 50 were selected from households and 50 were from the markets. About 54% and 46% of camel owners and milk sellers were males and females, respectively. About 51% of the participants were within the age ranged from 18 to 27, whereas about 72% were illiterate. Furthermore, 17, 16, 17 and 50 participants interviewed in the current study was selected from K/Bobasa, Riski, Agudora households and Boko market, respectively (Table 1).

Detection and enumeration of Staphylococcus aureus *in raw milk*

Of 156 milk samples collected, 38.5% of the milk samples had *S. aureus*, ranging from 2.76 to 5.1 log CFU/mL with a mean value of 4.83 log CFU/mL. Similarly, *S. aureus* was detected in the samples collected from the household and market that accounted for 36.4 and 42.3%, respectively. Furthermore, of the 60 *S. aureus* positive samples, 23 (38.3%) had *S. aureus* counts ranging from 4 to 5 log CFU/mL, which is above the recommended level for human consumption (Table 2).

Factors associated with Staphylococcus aureus *contamination*

At primary production sites, the study showed that the prevalence of *S. aureus* was found higher in animals of old age (>8 years), many parities (>7 calves) and the early lactation stage (1-4 months). The prevalence of *S. aureus* showed a statistically significant association (*P*-value of <.05) with respect to parity and age. There was a statistically significant association (*P*-value < .05) between the prevalence of *S. aureus* and the sources of milk and lactation stage (Table 3).

Hygienic practice and Staphylococcus aureus *contamination of milk*

Of the 50 camel milkers interviewed, 38% used well water for washing purposes during milking, whereas 72% used cold water only for washing milking buckets and their hands. About 96% of milkers did not practice hand and milking bucket washing between milking every camel. However, about 60% sold morning and evening milk separately, whereas 40% sold mixed morning and evening milk. Furthermore, the study found that there was statistically significant association

Table 3. Association between socio-demographic characteristics and prevalence of *Staphylococcus aureus*.

VARIABLES (N=50)	CATEGORY	SAMPLE SIZE	S. AUREUS PREVALENCE (%)	X^2 (*P*-VALUE)
Places/location	Bobasa	26	10 (38.4)	0.46 (.790)
	Riski	26	8 (30.76)	
	Agudora	26	9 (34.6)	
Age	Young	20	2 (10)	8.196 (.016)*
	Adult	30	11 (36.7)	
	>8y	28	14 (50)	
Parity	Few	20	2 (10)	8.338 (.014)*
	Mid	32	12 (37.5)	
	>7 calves	26	13 (50)	
Lactation stage	1-4mo	14	9 (64.3)	9.30 (.010)*
	Mid	39	14 (36)	
	Late	25	4 (16)	
Milking Order	Sequential	32	6 (18.75)	6.03 (.01)*
	Random	46	21 (45.65)	
Source of milk	Different herd	24	16 (66.7)	8.456 (.013)*
	Single herd	34	11 (32.4)	
	Single camel	20	6 (30)	
Condition of milk	Morning milk	30	16 (53.3)	2.43 (.12)
	Mixed morning and evening milk	48	17 (35.4)	

*Statistically significant.

(*P*-value < .05) between the prevalence of *S. aureus* and other variables such sources of water, and milking of camel with mastitis (Table 4).

Discussion

Of 156 milk samples collected, 38.5% of the milk samples had *S. aureus* that was in line with the study conducted in Egypt,[25] which reported 38.5%, and in Kenya,[26] which found about 34.9% prevalence of *S. aureus* in raw camel milk. However, the current study found a higher prevalence than studies conducted in Ethiopia,[19] Iran,[27] and Turkey[28] that reported 11.45%, 11%, and 10.2% positive samples, respectively. The variation might be due to the difference in the application of hygiene and safety measures in the study locations.

Furthermore, the present study found that 34.6% of milk samples collected from households were contaminated with *S. aureus*. This finding was consistent with the findings of another study conducted in Kenya,[29] which reported a mean count of *S. aureus* of 5 log CFU/mL at production and over 6 log CFU/mL at marketing..

The current study reported a higher prevalence than the finding of another study conducted in Somaliland,[30] which found 24.2%. However, the current study reported a lower prevalence of samples contaminated with *S. aureus* than another study conducted in Ethiopia,[31] and Pakistan,[32] which reported 54.3% and 48.15, respectively. The variation might be due to the variation in hygiene and safety practices, community awareness, and the prevalence of mastitis in camel herds.

Furthermore, the present study reported a 42.3% prevalence of *S. aureus* in milk samples collected from the market. The current study reported a higher prevalence of *S. aureus* than the findings of another study conducted in Ethiopia (15.62%).[19] The variation may be due to the difference in unhygienic practices of milk handling, use of poor quality water for cleaning of equipment, handling of milk at a high ambient temperature, long distance transportation from production to marketing without any cooling facilities, and bulking of milk from different herds.

In the present study, a mean count of *S. aureus* of 4.83 log CFU/mL was relatively in line with the findings of other studies conducted in Ethiopia[19] and Kenya[29] that reported the prevalence of 4.83 and 4 log CFU/mL, respectively. However, the current finding is higher than the finding reported by other studies conducted in Kenya,[33] Saudi Arabia[34] and United Arab

Table 4. Factors associated with *Staphylococcus aureus* contamination of milk samples, 2019.

VARIABLE (N = 50)	CATEGORIES	TOTAL (%)	X^2 (*P*-VALUE)
Sex	Female	3 (6)	2.02 (.36)
	Male	47 (94)	
Age	Young	20 (40)	0.26 (.88)
	Adult	30 (60)	
Education level	Primary	14 (28)	0.27 (.87)
	Illiterate	36 (72)	
Hand washing before milking	Yes	19 (38)	2.8 (.25)
	No	31 (62)	
Sources of water	Hand pump	11 (22)	7.6 (.02)*
	Well	39 (78)	
Milking of camel with mastitis	Yes	8 (16)	5.6 (.06)*
	No	42 (84)	
Milking order	Sequential	20 (40)	2.9 (.23)
	Random	30 (60)	
Hand washing between milking	Yes	2 (4)	4.4 (.11)
	No	48 (96)	
Washing buckets between milking	Yes	2 (4)	4.4 (.11)
	No	48 (96)	
Milking frequency (per d)	Twice	20 (40)	4.5 (.11)
	Once	30 (60)	
Milking time	Morning	30 (60)	4.5 (.11)
	Morning and evening	20 (40)	

*Statistically significant.

Emirates[35] which found 3.08, 3.86, and 3.08 log CFU/mL, respectively. The variation may be due to the difference in the implementation of hygienic practices such as handling of milk, methods of washing the equipment, temperature control, and personal hygiene.

In general, the current study found that, about 38.5% of the samples was contaminated with *S. aureus* of which 23(38.3%) were beyond the recommended level (4-5 log CFU/mL). This indicates poor hygienic practices during milking, the cleaning of the equipment, and storage condition. Furthermore, it represents that raw milk in the study areas was a potential health risk for humans.

The present study results showed that the prevalence of *S. aureus* was statistically significant (*P*-value < .05) among the age groups of animals. The prevalence of *S. aureus* among old, adult, and young animals was 50%, 36.7%, and 10%, respectively. Similarly, the prevalence of *S. aureus* has statistically significant variation (*P*-value < .05) among the parity numbers of animals, with the highest prevalence being recorded at 50%, 37.5%, and 10% in many, moderate, and few animals, respectively. This result is in line with the findings of a previous study conducted in Somaliland.[30]

Furthermore, this finding showed that the prevalence of *S. aureus* is statistically significant (*P*-value < .05) among the lactation stages of animals, with a high prevalence of 64.3%, 41%, and 20% in the early, mid, and late lactation stages, respectively. This is in line with the other studies conducted in Ethiopia[31] and Somaliland[30] which reported the early stage of lactation presented more prevalence of mastitis, which in turn increased the prevalence of *S. aureus* in raw camel milk. The difference may be due to animals' early stages of lactation having a lower resistance to immune system infections and being more susceptible to mastitis, which leads to an increased prevalence of *S. aureus* in raw milk.

Furthermore, the present study showed that there was a statistically significant association (*P*-value < .05) between the prevalence of *S. aureus* and the sources of milk that was in line with the finding of another study conducted in Kenya[33] reported the sources of milk as the risk factors for the contamination. Thus, effective implementation of risk-based food or milk safety by the concerned bodies, proper hygiene and safety practice,[36] training, and appropriate control measures are essential to protect the health of the consumers and the public as whole.[37]

Conclusions

The current study found that an overall prevalence of *S. aureus* was 38.5%. Similarly, the study found some risk factors associated with *S. aureus such as* hygienic practices. In general, more than one-third of milk samples were contaminated with *S. aureus* at levels higher than the maximum permitted level. The findings of this study suggest a potential risk of foodborne infection and intoxication. Therefore, implementation of adequate hygiene and safety practices is very important to prevent the consumption of contaminated fruit juices, which leads to foodborne illness.

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Author Contributions

IT conceived the idea and collected the data, and played a major role. The authors (DAM, DB, and SG) contributed to data analysis, writing, and editing the document. IT, DAM, DB, and SG gave valuable ideas for the manuscript and revised the manuscript. Finally, the authors read and approved the final version to be published and agreed on all aspects of this work.

Data Availability

All data are included in this study. However, additional data will be available from the corresponding author upon reasonable request.

Ethical Consideration

Ethical approval for this study was obtained from College of Veterinary Medicine, Haramaya University. Informed consent has been obtained from all individuals included in this study.

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