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Original Article

Pasteurella organism: Its isolation and identification from pneumonic lungs of goats in Ethiopia

Shimelis Mengistu Hailu[1], Dinaol Belina Kitila[1,#], Amare Eshetu Gemeda[1] and Mitiku Tarekegn[1]

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AFFILIATIONS

[1]College of Veterinary Medicine, Haramaya University, PO Box No 138, Dire Dawa, Ethiopia.

CORRESPONDENCE:

[#]Dinaol Belina Kitila,
College of Veterinary Medicine, Haramaya University, Ethiopia.
E-mail: belina.timketa@gmail.com

ABSTRACT

Objective: The objective of this study was to isolate and identify *Pasteurella spp.* associated with pneumonic lungs showing respiratory signs of goats in Ethiopia.
Materials and methods: A total of 2400 goats that were slaughtered at the Hashim's Ethiopian Livestock and Meat Export abattoir, Ethiopia were randomly selected for this cross-sectional study during the period of October 2013 to July 2014. Detail ante-mortem, and post-mortem (PM) lesions were inspected, and the suspected samples were collected aseptically from the lungs. Among 2400 goats, 31(1.29%) goats were not slaughtered because these goats showed severe clinical signs. Thus, 2369 goats were slaughtered finally. The collected samples were subjected for isolation and identification of bacterial species following conventional methods such as culture and biochemical examinations.
Results: Out of 2400 goats examined, 960(40%) animals showed different abnormal respiratory signs. Based on PM findings, 16.21% (n=384/2369) lungs were found as pneumonic, of which 78.38% (n=301/384) were found to be associated with *Pasteurella* organism. The overall prevalence of *Pasteurella* organism (*Mannheimia haemolytica* and *Pasteurella multocida*) was 12.71% (n=301/2369). In this study, youngers and goats with medium body condition score (BCS) had greater probability ($P<0.05$) to be infected by the bacteria though there was no difference in exposure to the organism among goats from Arsi, Bale and Hararghe. On the other hand, out of 301 positive cases, 274(91.03%) were caused by *M. haemolytica*, and 27(8.97%) were caused by *P. multocida* isolates.
Conclusion: *Pasteurella* organism especially *M. hemolytica* is one of the most common causes of pneumonic pasteurellosis in caprine at the study area. So, chemoprophylaxis needs to be given to small ruminants prior to transportation or other stress conditions.

KEYWORDS

Caprine; Isolation; *Pasteurella sp.*; Pneumonic lung

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INTRODUCTION

Africa has a population of 205 million sheep and 174 million goats representing approximately 17% and 31% of the world total small ruminants, respectively. In Ethiopia, 34-40 million of total African livestock population are found (FAO, 1994). However, sheep and goats produce only about 16% of the world's meat (CTA, 2003). Small ruminants play an important role in nutrition and income generation of people around the world. They serve primarily as source of meat, and also provide milk, skin, and wool (Mbilu, 2007). Owing to their remarkable adaptability to adverse environments, goats assume important position in Ethiopian livestock economy. In combination with sheep, they supply more than 30% of all domestic meat consumption, and generate income from exports of live animal, meat and skin (Aleme and Zemedu, 2015).

In spite of large livestock population in Ethiopia, the productivity remains marginal and this may be mainly due to malnutrition, prevalent diseases and management problems, poor genetic potentials of the local stock, marketing, social factors, structural constraints (Biffa et al., 2007). Among the diseases, Pneumonia reported as the most important infections and frequently diagnosed in veterinary clinics and abattoirs in goats in Ethiopia. Pneumonia is one of the most common respiratory problems in small ruminants throughout the world. In goat herds, pneumonia increases production costs associated with expensive treatments. Although pneumonia often occurs in kids, illness and deaths also occur in adult animals (Ramírez-Romero and Brogden, 2000). Bacterial infection of the respiratory tract may be primary, occurring in healthy individuals or secondary to a large number of conditions which depress resistance. Secondary bacterial infection occurs especially when the bacterial growing in the nose and throat extends downwards, usually giving a mixed infection (Megra et al., 2006). According to Ramírez-Romero and Brogden (2000), the most frequent causes of respiratory infection and death are *Pasteurella multocida* or *Mannheimia haemolytica* (previously called *P. haemolytica*). *P. multocida* and *M. haemolytica* are commonly found in the upper respiratory tract of healthy goats. *M. haemolytica* are subdivided into two groups; A and T. Type A is most prevalent and is associated with a severe form of pneumonia. Pasteurellosis broadly refers to any of the disease conditions caused by species of the genus *Pasteurella* (Davies et al., 2003). Pneumonic pasteurellosis is an acute infectious disease that causes widespread financial losses because of death, reduced live weight, delayed marketing, treatment costs and unthriftness among survivors (Aielo, 1998; Ozbey et al., 2004). *P. haemolytica* is the bacterium that is frequently isolated from shipping fever, which affects sheep and goats of all ages worldwide (Ozbey et al., 2004), whereas *Mannheimia sp.* naturally inhabits the upper respiratory system particularly in tonsils and nasopharynx of healthy sheep and goats and other wild and domestic animals (Chen et al., 2002).

The disease 'shipping fever' is resulted when an animal is compromised by any of a variety of stress factors as changing weather, shipping (transportation), malnutrition, bacterial invasion of host defense, viral infections, nasopharyngeal colonization and dehydration (Radostits et al., 1994; Aielo, 1998). Various *M. haemolytica* virulence factors influence the outcome of bacterial-host interactions (Singh et al., 2011). Capsular serotyping provides the primary basis for the classification of strains and epidemiological typing of *M. haemolytica* (Peterson et al., 2001). Furthermore, the purified organism is subsequently classified according to phenotypic traits such as morphology, carbohydrate fermentation patterns and serological properties.

In Ethiopia, Pasteurellosis is a common respiratory infection and economically significant causing outbreaks of acute pneumonia in sheep/goats of all ages that may end with death of sheep and goats (Marru et al., 2013). However, very few studies have been conducted on identification and isolation of *Pasteurella spp.* from pneumonic lesion at abattoir in goats. Therefore, the general objective of this study was to determine prevalence of *Pasteurella spp.* in goats slaughtered at Hashim's Ethiopian Livestock and Meat Export (HELIMEX) abattoir and in goats with pneumonic lesions at the abattoir in bishoftu, and finally to isolate and identify *Pasteurella spp.* involved in pneumonic lesion of the slaughtered caprine species.

MATERIALS AND METHODS

Ethical approval: The study was approved by the Institutional Review Boards of the Addis Ababa University. Informed consent was obtained from study participants as it is there are no procedures in the study that suffers animals/against animal welfare.

Study area: The study was conducted in Bishoftu town at Hashim's Ethiopian Livestock and Meat Export (HELIMEX) abattoir, East Showa zone of Oromia regional state. The area is located at 9^0N and 40^0E with an altitude of 1880 meter above sea level in the central highland of Ethiopia at 47 Km South East of Addis Ababa (NMSA, 2006). Currently the abattoir is one of the most facilitated modern export abattoirs in Ethiopia and is exporting meat of small ruminants. During the study the average number of sheep and goats slaughtered at this

Table: 1. Description of the study areas

Geographic origin	Altitude (m.a.s.l.)	Latitude	Average annual rainfall (mm)	Aver. max and min daily temperature (°C)	Production system
Arsi	1800 to 4130	09°36'N 39°08'E	2000-4000	20-30 and 12-14	Crops and livestock
Bale	1500 to 4250	6°55'-7°70'N & 40°10'E	400-1400	20-25 and 10-15	Crops and livestock
Hararghe	1000 to 3405	8°29'59.99"N 40°39'59.99E	700-1200	23 -24.4 and 9.6-11.2	Mixed cash crops and Livestock

Sources: (CSA, 2013; NMSA, 2006)

abattoir per day were 1000 and 1200, respectively. The small ruminant animals slaughtered at the abattoir were purchased from different parts of the country particularly, Hararghe, Arsi and Bale zones and transported by vehicle. Therefore, animals were encountered different ecological areas and management conditions at their origin which is described in **Table 1**.

Study population: Study was conducted on caprine slaughtered at Hashim's Ethiopian Livestock and Meat Export (HELIMEX) abattoir with discrimination of their origin, body condition and age.

Study design: Cross-sectional study was undertaken from October 2013 to July 2014 to determine prevalence of pneumonic lesions and identify *Pastereulla spp.* from pneumonic lesions in goat slaughtered at the abattoir.

Sample size determination: The sample size was determined by the formula described by Thrusfield (2005), at 95% confidence level and 5% precision, and considering no previous such study report from the abattoir. Accordingly, a total of 384 goats were included in the study. However, to maximize the precision sample size was increased by 6.25 folds and a total of 2400 goats were included.

Sampling procedure and processing: The abattoir was visited 3 times per week and on average 20 animals was included in the study per day using systematic random sampling method for ten (10) months. After such selection animals were grouped in to young and adult according to Steele (1996) and in to good, medium and poor body condition scores (Belina et al., 2012). During ante-mortem (AM), each goat was then came into general physical examination and data regarding current clinical manifestation of diseases, giving special attention to the respiratory system was recorded in line with origin, age and body condition of study animals. Immediately after slaughter of those with respiratory signs, lung tissue of different sizes and sometimes the whole lung showing pneumonic lesions (from visual examination) were collected using sterile forceps and scalpel blade. The lung specimen was then placed separately in sterile plastic bags kept in ice box.

Then detailed post-mortem (PM) examination was made by visual examination and through palpation of air ways, lung and the corresponding bronchial lymph nodes for the presence of any lesion. The gross appearance, location and size of the lesions were also recorded. An incision was also made for further observation and any abnormality was recorded. Finally, the sample was transported to National Veterinary Institute (NVI) for bacteriological examination.

In the laboratory pneumonic lung sample were processed for isolation of bacteria. To avoid surface contaminants, the surface of the lung tissues was seared with a hot spatula (Quinn et al., 1999), and exudates was collected from the interior portion using sterile pasture pipette through the seared surface. In case when there was no exudates, small pieces of lung tissue collected from the sterilized surface of the sample with the help of sterile forceps and scalpel blade was inoculated in to sterile screw capped test tube with 5 mL of brain heart infusion (BHI) broth and blood agar base(BBL®). The inoculated broth tube was incubated aerobically at 37°C for 24 h. After 24 h of incubation, a loopful broth culture was platted on to the sheep blood agar (BBL®, Becton Dickison, USA) by quadrant streaking method and incubated aerobically at 37°C for 24 h (Sisay and Zerihun, 2003).

After 24 h of incubation, the plates were observed for the growth of the bacterial colonies. The size and morphology of the colony, pigment production, presence of hemolysis and the type of hemolysis were observed and recorded. Then, the isolated colonies were sub-cultured by half platting on blood agar and MacConkey agar (Oxoid, Basingstroke, England), and incubated at 37°C for 24 to 48 h. Then, a single colony was sub-cultured on BHI agar and Blood agar, and incubated for 24 h at 37°C. after obtaining pure colonies, primary and secondary identification tests were conducted according to the standard technique recommended by Quinn et al. (1999) and Fekadu (2005).

Data analysis: The data was entered to Microsoft excel spread sheet and the data was analysed using SPSS version 20. Descriptive statistics was also used to summarize the data. The prevalence of *Pasteurella sp.* was calculated as the number of positive isolates divided by the total number of animals' lung positive for pneumonic lesions. *Chi*-square was used to test the association among study variables.

RESULTS

The current study result indicated, out of 2400 goats examined during AM inspection 960(40%) were recorded as those showing different abnormal respiratory signs, among these 31(1.29%) goats were not slaughtered. Coughing, fast and shallow breathing, fever, nasal discharge and open mouth breathing were the abnormal respiratory signs observed at AM inspection (**Table 2**).

At AM inspection, a total of 960 goats showed different abnormal respiratory signs; however, only 384 caprine (66, 132 and 186 from Arsi, Bale and Hararghe, respectively) were found to be positive for pneumonia at PM examination. Hyperemic, congestion and different exudates were some of the pathological lesions contributed to the pneumonic lesions developments (**Figure 1**).

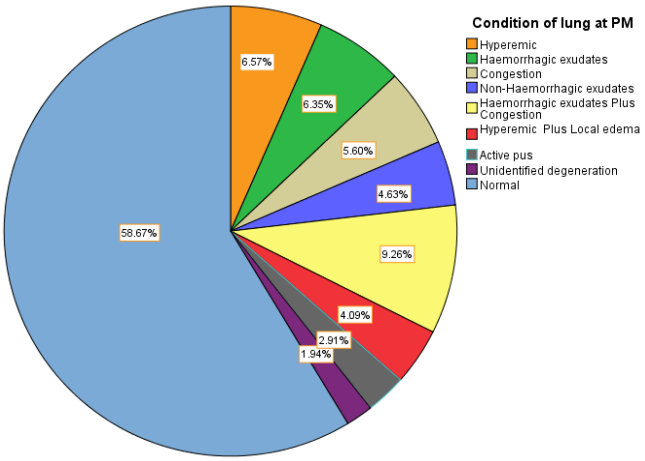

Figure 1: Different pneumonic lesions examined at post-mortem examination.

Pasteurella spp. are normally found in the upper respiratory tract of healthy livestock and domestic animal species. However, isolation of this organism from lower respiratory tract usually indicates a disease condition. The present study indicated the overall prevalence of *Pasteurella spp.* (*M. haemolytica* and *P. multocida*) was 12.71% (n=301/2369) whereas prevalence of *Pasteurella spp.* considering only pneumonic lungs was 78.38% (n=384). In this study, youngers (54.48%) and goats with medium body condition score (59.80%) statistically had greater probability ($P<0.05$) to be infected by *Pasteurella* organism. Though statistically not significant, higher prevalence of pastereulosis was found in goats from Hararghe 141(46.84%) than in those from Arsi and Bale zones (**Table 3**). On the other hand, out of 301 positive tests, 274 (91.03%) were *M. haemolytica* isolates whereas 27 (8.97%) were *P. multocida* isolates (**Table 4**).

DISCUSSION

The current study result indicated, out of 2400 goats examined during AM inspection 960(40%) were recorded as those showing different abnormal respiratory signs such as coughing, fast and shallow breathing, fever, nasal discharge and open mouth breathing. In agreement to the present study Mashishi (2007), also stated mucous or pus coming from nostrils, coughing, fast and difficult breathing after exercise, fever, depression and weight loss are signs appear in few days after the animal exposed to stress. In this, the lungs of 31(1.29%) goats did not examined during PM inspection because these goats showed severe clinical signs and detained from being slaughtered. Attributing to the current study FAO (1994) also explained abnormalities in respiration, behavior, gait and posture, growths on the eye, discharges from the nose, excessive saliva from the mouth and etc. are signs of diseases and abnormalities and animals showing such clinical signs should be held for veterinary examination and judgment.

It is worth mentioning that *M. haemolytica* and *P. multocida* are commensally present as normal constituents of the nasal and pharyngeal microflora of healthy ruminants. However, isolation of this organism from lower respiratory tract usually indicates a disease condition (Mohamed and Abdelsalam, 2008). Ugochukwu (2008) explained in his review that different scholars are also experimentally confirmed that under certain conditions associated with debilitation, nutrition and climatic factors, this organism may singly or in concert with other organisms flare up to cause severe infections with high morbidity and mortality. The *Pasteurella* organism can be considered as etiology for pneumonic pasteurellosis (pneumonic lesion development) and can also easily initiate infection in the body in man as well as in animals because of its toxigenicity that have deleterious effects on organs systems and immuno-responsiveness. In such cases there is chance mixed infection occurrence (with other bacteria and viruses) which in turn makes *Pasteurella* organism a highly invasive, pathogenic and virulent. Yesuf et al. (2012) also isolated *Staphylococcus*, *Streptococcus* and other bacterial species from pneumonic lungs in

Table 2. Proportion of goats showed different respiratory signs examined at Ante mortem investigation (N=960)

Condition at AM	No. of goats showed the sign (%)	Judgement
Coughing only	127(5.29)	CPFS
Fast and shallow breathing	125(5.20)	CPFS
Moderate fever only	118(4.91)	CPFS
Coughing plus nasal discharge	109(4.54)	CPFS
Nasal discharge only	151(6.29)	CPFS
Coughing plus moderate fever	103(4.29)	CPFS
Coughing, moderate fever plus discharge	93(3.87)	CPFS
Fast breathing plus moderate fever	84(3.5)	CPFS
Open mouth breathing	19(0.79)	CPFS
High fever plus coughing	31(1.29)	Detained
Total	960(40)	-
Normal	1440(60)	Passed
Ground total	**2400(100)**	

CPFS= Conditionally passed for slaughter

Table 3. Prevalence of pasteurellosis in pneumonic lung by age, animal Origin and body condition of goats at HELIMEX abattoir, Bishoftu.

Variables		Total number of goats (n)	Pneumonic lungs (n)	*Pasteurella* positive n (%)	χ^2 (*P*-value)
Age	Young	976	196	164(54.48)	6.608(0.01)
	Adult	1424	188	137(45.51)	
Origin	Arsi	438	66	54(17.94)	1.476(0.48)
	Bale	1045	132	106(35.21)	
	Hararghe	917	186	141(46.84)	
BCS	Good	1043	154	113(37.54)	37.065(0.00)
	Medium	1253	207	180(59.80)	
	Poor	104	23	8(2.657)	
Over all		2400	384	301(78.4)	

Table: 4. Frequency of *Pastereulla spp*. isolated from cultured sample (n=384)

Isolates	Frequency (%)
Manehemia haemolytica	274(91.03%)
Pastereulla multocida	27(8.97%)
Total	301(78.38%)

small ruminants. In our current study, PM inspection result showed a total of 384(16.21%) goats (66, 132 and 186 from Arsi, Bale and Hararghe, respectively) were found to be positive for pneumonia. In sheep and goats the lungs are easily infected if there are stress factors like extreme weather conditions, dipping, deworming, and mixing of animals from different places and age groups (Mashishi, 2007). Hence, since in our study, different age and sex groups of animals even different species were transported from different zones (in average not from less than 450 Km), mixing of different animals and transportation stress may expose goats in to lung infection (pneumonia). Transportation of the study animals in to a new geographic area may also be a source of stress in this study. Hyperemia, congestion and different exudates were also some of the pathological lesions characterizing the pneumonic lesions observed during the present study. Tijjani et al. (2012) reported that gross lesions investigated in pneumonic lung due to bacteria can be suppurative bronchopneumonia, granulomatous pneumonia, exudative pneumonia and consolidation. Ramírez-Romero and Brogden (2000) also explained a necropsy of lobes from the lungs of goats died of pneumonia pasteurellosis showed hemorrhagic (bloody) secretion, and possibly pus and dead tissue lesions.

The respiratory infections of *M. haemolytica* and *P. multocida* are associated with poor management practices, occur as a secondary infection, or occur as a consequence of severe stress. These pathogens can also cause outbreaks of acute pneumonia in goats of all ages (Ramírez-Romero and Brogden, 2000). Similarly, there was high prevalence of pasteurellosis in both young and adult goats included in the current study, though the prevalence was significantly higher in youngers (*P*=0.01) than in adults. Alemneh and Tewodros (2015) also reported the prevalence of pasteurellosis is higher in youngers than in adults. Marru et al. (2013) also explained pneumonic pasteurellosis occur in all ages of sheep and goat with, the most susceptible in lambs and kids during first life, and dam at lambing. This might be due to the immune status of the animals being able to predispose to the bacterial infection and other pre-disposing etiological agents.

In the current study, there was no significant difference (P>0.05) in the prevalence of pneumonic pasteurellosis among geographical origins of the study animals; though the highest percentage (46.84%) was found in goats from Hararghe areas. On the other hand, there was statistically significant differences (P=0.00) in occurrence of pasteurellosis among body condition score of goats; where the highest prevalence (59.80%) was found in goats with medium body condition. This might be due to differences in number (nonproportional sampling was used) of goats included in this study.

M. haemolytica and *P. multocida* are the most frequent causes of respiratory infection and even death in goats are commonly found in the upper respiratory tract of healthy animals (Ramírez-Romero and Brogden, 2000). Similarly, in the present study, both *M. haemolytica* and *P. multocida* were isolated from the pneumonic lungs (lung lesions) with 78.38% (n=384) total prevalence. However, the current prevalence is in contradiction to the report of Alemneh and Tewodros (2015), who found 21.9% overall prevalence in goats from Fogera Woreda, Ethiopia.

According to the current results, *M. hemolytica* was the most frequently isolated isolates accounting 91.03% whereas *P. multocida* was revealed to be only 8.97%. This finding is partially consistent with the findings of Alemneh and Tewodros (2015), who reported 79.5% prevalence of *M. hemolytica*. On the other hand, the prevalence of *M. hemolytica* isolates was higher unlike the earlier report of Mohammed (1999) and Tesfaye (2004) from lung infection. The higher isolation rate found in this study might also be attributed to the quantity of pneumonic lung samples, which are large and incomparable with those studies. These bacteria have also been reported by different workers from different anatomical sites and animals. However, their loads vary from site to site and/or animal to animal. Mohammed (1999) and Tesfaye (2004) also reported this bacteria from caprine lung affected by pneumonic; other scholars Ugochukwu (2008) and Mohammed (1999) were isolated it from caprine and ovine pneumonic lungs. In addition, Shemsedin (2002) also reported it from camels' lung. The invariable isolation of these organisms from pneumonic lungs of various animal species might indicate the significance of *Pasteurella* organism in different respiratory syndromes. The higher prevalence of *Pasteurella* organism in our case (78.38%) mighty be due to transportation stress plus stress overcrowded animals on the vehicle that can exacerbate the condition and even can lead to outbreaks. According to Caswell (2014), stress factor with or without viral infections interact to suppress the muco-ciliary clearance mechanism, which allow the proliferation of commensal bacteria in the respiratory tract. The isolation of *Pasteurella spp.* especially *M. haemolytica* as one of the common isolates of caprine pneumonic lungs confirms the assertion that both in terms of infection intensity and pathogenicity, *M. haemolytica* assume greater prominence in caprine pasteurellosis (Sisay and Zerhun, 2003).

In spite of the low percentage frequency (8.97%) isolation of *P. multocida* in this study, attention is drawn to the pathogenic potential of this organism in goats; which is in agreement with previous reports of Mohammed (1999) and Tesfaye (2004). *P. multocida* is a potential pathogenic bacterial organism, which has been incriminated, in both human and animal infections where it causes of ten times severe respiratory abnormalities that can terminate in death (Ugochukwu, 2008). Although, it may be found occasionally as normal inhabitant of the respiratory system, experimentally, evidence has shown that under certain condition associated with debilitation, nutrition and climatic factors, this organism may singly or in concert with other organism, flare-up to cause severe infection with high morbidity and mortality (Ugochukwu, 2008). The successful isolation of *P. multocida* in this investigation is, therefore, interesting not only because of its traditional role as a disease causing aerobic bacteria but also because of its toxigenecity as forwarded by Hall et al. (1987). Its toxin has been reported to have deleterious effect on organ system and immune responsiveness (Ugochukwu, 2008). Although the percentage isolation was relatively low (8.97%), the possible role of *P. multocida* as aetiology and pathogenesis of caprine pneumonia should not be under estimated.

Comparing the two *Pasteurella spp.*, *M. hemolytica* constituted 91.03% of the total pneumonic lungs (n=384), indicated that *M. hemolytica* was the major causative agent involved in caprine pneumonic lungs. This is in consistent with the previous reports of Tesfaye (1997), Mohammed (1999) and Ugochukwu (2008). *M. hemolytica*, the normal flora of upper respiratory tract, interferes with host defence mechanism favoring the multiplication of *Pasteurella spp.*, leading to bronchopneumonia in purely pneumonic animals (Aielo, 1998).

CONCLUSION

Goat constitutes the second major component of livestock in Ethiopia. However, efficient utilization of this potential resource is hampered by combination of health problem, poor management and feed shortage. Pneumonic pasteurellosis is one of the major diseases of goats in the area. This study reveals that pneumonic pasteurellosis is the major disease of goats in the area and entails substantial loss. *M. haemolytica* is the most common

cause, whereas *P. multocida* is rare. Young animals are mostly susceptible to pasteurellosis. Measures such as, improved managemental practices, minimizing transportation stress, providing good quality hay and water and supplement should be taken. Vaccine against *P. multocida* type A is suggestive for the control and prevention of caprine pasteurellosis in the study area.

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CONFLICT OF INTEREST

The authors declare no conflict about interest.

AUTHORS' CONTRIBUTION

SM designed the work and conducted the experiments. MT participated in data collection. DB participated in analysis and interpretation of the data. AE drafted the manuscript. All the authors read and approved the final version of manuscript.

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