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Prevalence and antimicrobial susceptibility of *Salmonella* species from lactating cows in dairy farm of Bahir Dar Town, Ethiopia

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Salmonella is the most important disease causing bacteria in persons as well as in animals. Antimicrobial-resistant *Salmonella* can be transmitted from animals to humans through consumption of contaminated food and food products. Thus, this study was conducted to determine the prevalence and antimicrobial susceptibility of *Salmonella* species from lactating cows in dairy farms of Bahir Dar town. A cross-sectional study was conducted in Bahir Dar town from November 2012 to June 2013. Identification of *Salmonella* spp. was made biochemically with Kligler Iron agar (KIA), Urea agar, Sulfur Indole Motility (SIM), Lysine Deoxycholate agar (LDC) and Simmons citrate agar. Antibacterial sensitivity of the isolates was tested using the Kirby-Bauer test. Thus, out of the total 384 milk samples collected, 36 (9.35%) were positive for *Salmonella* spp. *Salmonella* isolates in this study were highly resistant to ampicillin (94.4%) followed by tetracycline (52.8%) and trimethoprim-sulfamethoxazole (38.9%). However, the isolates showed high susceptibility to fluoroquinolones (norfloxacin and ciprofloxacin) at 100 and 93.9%, respectively followed by gentamicin (94.44%). Likewise, the *Salmonella* isolates showed 72.22% of multidrug-resistance (resistance to two or more antibiotics) in the study area. In conclusion, the incidence of multidrug-resistant *Salmonella* spp. isolated from milk of lactating cows was high in the study area. Furthermore, the study also revealed the high rate of drug resistance pattern to commonly used antibiotics among the isolated *Salmonella* spp.

Key words: Antimicrobial susceptibility, lactating cows, dairy farm, *Salmonella*.

INTRODUCTION

Dairy cattle are a fundamental reservoir for *Salmonella* and have been associated in case of human salmonellosis. The United States National Animal Health Monitoring System Dairy 96 study reported 5.4% of milk cows shed *Salmonella* and 27.5% of dairy operations had at least one cow shedding *Salmonella*. *Salmonella* has

been isolated from all ages of dairy cattle and throughout the production process (Edrington et al., 2008).

Ethiopia has a major national livestock resource, which is a vital part of farming production system (Mesele et al., 2012). Alongside it has the biggest beef population of any African country with an estimated 35 million tropical

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livestock units (TLU); this includes 31 million cattle, 42 million sheep and goats, 7 million equines, 1.2 million camels, more than 53 million chickens and fishery resources (Temesgen et al., 1999). Cows signify the leading share of cattle population of the country. According to the Food and Agriculture Organization, 42% of the total cattle heads for the private holdings are milking cows (FAO, 1990).

Raw milk remains an ideal growth medium for microorganisms (Haridy, 1992). The frequency of both small and large-scale outbreaks of illness is attributed to milk borne. Hence, the presence of microbes such as bacteria and fungi into milk can multiply and bring about spoilage making raw milk which makes it inappropriate for human intake due to rancidity, musty odors or toxin production. Moreover, the type and level of microorganisms in the milk is influenced by animal health, the farm environment and production methods. The main microbiological hazards associated with milk and dairy products include *Salmonella*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Enterobacter sakazakii*, *Brucella*, *Campylobacter*, *Mycobacterium bovis*, *Escherichia coli*, *Shigella*, *Streptococcus pyogenes*, and *Yersinia enterocolitica* (Trynness et al., 2012).

Salmonella is one of the utmost main causal agents of foodborne illness (Gorman and Adley, 2004). Contamination of raw milk and products with *Salmonella* species is typically due to infected persons and contamination of the environment, since natural infections of the udder are rare and seldom contribute to human food poisoning. Poor sanitation in dairies, particularly those from developing countries, has often been considered as one of the main reasons for contamination of milk with both spoilage and pathogenic bacteria. Contaminated foods are often animal origin, such as beef, poultry, milk, or eggs but any food, including fruits and vegetables, may become contaminated (Mohammad et al., 2011). But the dissemination of *Salmonella* serotypes among cattle varies greatly over time and differs among geographic regions, age groups, clinical manifestation and production systems (Hoelzer et al., 2011).

The introduction of *Salmonella* into a dairy farm can occur through a variety of routes, including purchased cattle, contaminated feed or water, wild animals such as rodents and birds, human traffic, and insects (Kevin et al., 2010). However, on farm management practices can help control spread of foodborne pathogens in dairy cattle. Initial quarantine of new animals, proper nutrient management, good hygiene, and access to fresh clean water are some of the practices that can reduce the possibility of introducing or increasing the prevalence of *Salmonella* within a dairy herd (Kabagambe et al., 2000).

Currently, antimicrobial resistance of *Salmonella* is becoming a worldwide issue. The extensive management of medically important antibiotics to food animals at sub therapeutic or prophylactic doses may stimulate on

farmhouse selection of antimicrobial resistant strains and distinctly raise the public health threats linked with feeding of contaminated meat products (D'Aoust et al., 1992). In developing countries, antimicrobial agents are broadly used in both animal and human health practices and a high level of antimicrobial resistance in *Salmonella* has been reported (Abebe et al., 1997). Hence, treatment with antimicrobials is crucial for the proper management of severe or invasive human salmonellosis (Kayode et al., 2010).

The prevalence of salmonellosis both in humans and animals is problematic to assess because of lack of an epidemiological surveillance system in place, which is particularly true in developing countries. In Ethiopia, few studies were conducted on the presence of *Salmonella* spp. in lactating cows in dairy farm. However, the evidence of salmonellosis in lactating cows is very limited (Zellalem et al., 2011; Fufa et al., 2017). It is known that the incidences of *Salmonella* spp. from cows is different in different places due to the geographical location, season, farm size, number of animals on the farm, farm management practices, like housing conditions, feeding habits, types of feed given for the cattle. Hence, research on prevalence of *Salmonella* spp. in lactating cows plays a great role. So, updated information on their prevalence and antimicrobial susceptibility is very important for proper selection and use of antimicrobial agents in a setting. In order to improve milk quality and quantity and public health at all levels of dairy farms, different activities should be applied. Therefore, the present study was undertaken to determine the prevalence and antimicrobial susceptibility of *Salmonella* spp. from lactating cows in dairy farm of Bahir Dar town. So, this research finding provides information on the present status of lactating cows.

MATERIALS AND METHODS

Study area and subjects

This study was done in Bahir Dar town. Bahir Dar is found in North-Western Ethiopia approximately 578 km from Addis Ababa, having altitude and longitude of 11°36'N37°23'E. The town has an elevation of 1840 m above sea level. Based on the 2011 Population and Housing Census of Ethiopia, the total number of its population is 256,999 (CSA, 2011). All lactating cows which were used for milk production in the dairy farm in Bahir Dar were included in the study.

Research design

A cross-sectional study was conducted in Bahir Dar town from November 2012 to June 2013. Checklist was prepared and used to observe milking process, condition of bedding, storage of milk washing equipment and washing hands.

Sample size determination

In estimating the sample size, the minimum number of the sample

size (N) was determined using statistical formula of sample size calculation (Daniel, 1999).

$$N = z^2 p (1-p)/d^2$$

where N is the minimum sample size required, z is 1.96 at 95% confidence interval, d is margin of sampling error tolerated (5% marginal error was used), p is an estimate of the prevalence rate for the population, since the overall prevalence of the study area was not known p was taken to be 50% for the calculation.

Therefore, the minimum number of the sample size, $N = (1.96)^2 \cdot 0.5(1-0.5) / (0.05) = 384$.

Sample collection

A total of 384 milk samples were collected from the selected dairy farms in Bahir Dar town from December 2012 to February 2013. The samples were carefully collected using sterile test tubes which were then packed to avoid any possibility of leakage or cross-contamination. Individually identified containers (test tubes) were placed in icebox and packed enough. Immediately after collection, samples were transported to the post graduate microbiology laboratory in Bahir Dar University and stored in refrigerator until microbiological analysis.

Isolation of *Salmonella* spp.

The isolation of *Salmonella* spp. was done at Bahir Dar University Post Graduate Microbiology Research Laboratory. One milliliter of milk sample was pre-enriched in 9 ml of buffered peptone water. The pre-enrichment broth after incubation was mixed and a portion (0.1 ml) of the pre-enriched culture was transferred into a tube containing 10 ml of Selenite Cysteine (SC) broth and incubated at 37°C for 24 h. After incubation, a loop-full of selective enrichment was transferred and streaked onto the surface of Xylose lysine deoxycholate (XLD) agar and incubated at 37°C for 24 h. Typical colonies of lightly transparent zone of reddish color with/without black color at the center were picked and streaked onto nutrient agar for purification and incubated at 37°C for 24 h. A single colony of bacteria was taken from the Nutrient Agar and inoculated into Tryptic Soy Agar slant. The slant was incubated at 37°C for 24 h. Identification of *Salmonella* spp. was made biochemically with Kliger Iron agar (KIA), Urea agar, Sulfur Indole Motility (SIM), Lysine Deoxycholate agar (LDC) and Simmons citrate agar (Cheesbrough, 2004).

Antimicrobial susceptibility testing

Antibacterial sensitivity test was performed according to the Clinical and Laboratory Standards Institute (CLSI, 2006) using Kirby-Bauer disk diffusion test on Muller-Hinton agar medium (Oxoid England) (Bauer et al., 1966). The isolates were tested for the following antibiotics include ampicillin (AMP, 10 µg), gentamycin (CN, 10 µg), chloramphenicol (C, 30 µg), tetracycline (TE, 30 µg), ciprofloxacin (CIP, 5 µg) cefoxitin (FOX, 30 µg), norfloxacin (NOR, 10 µg), Nalidixic acid (NA, 30 µg) all from Oxoid, England and cotrimoxazole (Thrimethoprim/sulfamethoxazole, SXT, 25 µg) (Micromaster, India). *Escherichia coli* ATCC 25922 was used as quality control.

Data analysis

Laboratory results were entered into a computer. The data were analyzed using SPSS version 16 software. And a *p*-value less than

0.05 were considered as statistically significant. The Chi-square test was utilized to assess significant differences in antibacterial susceptibility of *Salmonella* spp.

Ethical considerations

Permission from owners of the dairy farm of Bahir Dar town for milk samples were obtained before milk sample collection.

RESULTS AND DISCUSSION

A total number of 384 milk specimens were collected from lactating cows from the dairy farms in Bahir Dar, Ethiopia. In addition to this, 50 complete checklists were collected from the owners. These checklists were designed, developed and used to collect milking process data and handling practice that were considered relevant to this study to see the association of these important variables with salmonellosis.

Prevalence of *Salmonella* spp. in the milk sample

From the total of 384 milk samples collected for the analyses of *Salmonella* spp., only 36 (9.35%) were positive for *Salmonella* spp. (Figure 1). The 95% confidence interval (CI) for the prevalence of *Salmonella* spp. among lactating cows was between 5.1 and 13.6%.

In this study, the occurrence of *Salmonella* spp. from lactating cows was 9.35% (5.1 to 13.6%, at 95% confidence interval). This result is in agreement with the prevalence of 10.76 and 10.5% *Salmonella* spp. isolated from the study on lactating cows, Addis Ababa and Modjo town, Ethiopia, respectively (Zellalem Addis et al., 2011; Fufa Abunna et al., 2017). Nevertheless, most of the studies are on slaughtered cattle from abattoirs and ready to eat food items (Alexander et al., 2009), the current study is comparable with the studies on prevalence of *Salmonella* spp. 11.5 and 7.1% conducted in Kombolcha and Debre Zeit, Ethiopia (Minte et al., 2011; Daniel et al., 2003), respectively. This study is in agreement with the report of Blau et al. (2004) who indicated that prevalence of *Salmonella* spp. was recorded in 7.3% in Dairy Operations in the United States (USA). Nevertheless, the prevalence of *Salmonella* spp. in the present study is upper than the frequency of *Salmonella* spp. isolated from dairy farm in Asella, Ethiopia, 4.4% (Takele et al., 2016) and slaughtered cattle and retail beef in Hawassa, Southern Ethiopia, 2.7% (Kokeb et al., 2017).

This variation might be attributed to numerous factors, such as topographical location, period, size of the farm, environment sanitation, farm management practices, variation in types of samples evaluated and differences in detection methodologies used. However, in spite of the variation, all of these studies proved quite clearly that milk can be a significant source of foodborne pathogens



Figure 1. Total numbers of lactating cows with and without *Salmonella* species in dairy farm of Bahir Dar, Ethiopia, 2013.

of human health significance (Nagal et al., 2006). In line with this, Akoachere et al. (2009) in Cameroon reported a higher prevalence (27%) of *Salmonella* among cattle than the current study.

Antimicrobial susceptibility testing

A total of 36 *Salmonella* isolates (N=36) were tested against nine commonly used antimicrobials, including ampicillin (AMP, 10 µg), gentamycin (CN, 10 µg), chloramphenicol (C, 30 µg), tetracycline (TE, 30 µg), ciprofloxacin (CIP, 5 µg) cefoxitin (FOX, 30 µg), norfloxacin (NOR, 10 µg), nalidixic acid (NA, 30 µg) and cotrimoxazole (thrimethoprim-sulfmethoxazole) (SXT, 25 µg) (Oxoid). The result of antimicrobial vulnerability pattern of *Salmonella* isolates is shown in Table 1.

The uppermost resistance was known for ampicillin 34 (94.4%) which is similar with the study conducted in Addis Ababa (Zelalem et al., 2011) and Harrer (Ayalu et al., 2011) followed by tetracycline 19 (52.8%) which is in agreement with the reported findings in Addis Ababa, Ethiopia (Zelalem et al., 2011). This might be due to the use of this antibiotic for long period of time in the community because it is relatively cheap and easily available.

The isolated *Salmonella* spp. tested had also resistance against chloramphenicol 13 (36.11%) which is similar with the report presented in Bahir Dar, Ethiopia (Bayeh et al., 2010). In line with this, the isolated *Salmonella* spp. tested had also resistance against some other antimicrobial agents like cotrimoxazole (trimethoprim-sulfamethoxazole) 14 (38.9%) and cefoxitin 11 (30.6) which is in agreement with studies in Debre-Zeit and Addis Ababa, Ethiopia (Bayleyegn et al., 2003a) and in sub-Saharan Africa. This is maybe due to unselective and prevalent uses of the frequently existing antimicrobials both in the veterinary and public health practices since, in these countries, people have easy access to various antimicrobials and can purchase them

without prescription (Leegaard et al., 1996). Thus, the present report contradicts with the study of Forough et al. (2012), which indicated that *Salmonella* isolates from Iran showed that *Salmonella* were resistant to chloramphenicol (21.42%), ampicillin (42.58%), and tetracycline (42.58%). This might be due to the difference in the study area. And also antibiotic resistant of *Salmonella* spp. increases regularly (Kariuki et al., 2006). The previous drugs such as chloramphenicol, ampicillin and trimethoprim-sulfamethoxazole are occasionally used as alternatives (Cui et al., 2009).

As shown in Table 1, all the 36 *Salmonella* isolates were detected being the highest level of susceptibility to norfloxacin (100%) followed by ciprofloxacin (97.1%) and gentamycin (94.44%) and less sensitive nalidixic acid (86.1%). This is also comparable with the result conducted in USA (Blau et al., 2004). In line with this, the result of these antimicrobial agents, like ciprofloxacin and gentamycin, is covenant with the study done in Asella town, Ethiopia (Takele et al., 2016) and norfloxacin, in Harrer (Ayalu et al., 2011) and in Sudan (Fadlalla et al., 2012). This would probably be due to the fact that the drugs are relatively expensive and newly introduced, compared to the other commonly used antibiotics. Regarding to nalidixic acid, this study contradicts with the studies conducted in Nigeria (Kayode et al., 2010) and in Ethiopia (Daniel et al., 2003). Unlike these results, a high level of resistance to gentamycin (75.6%) was stated from Gondar, Northwest Ethiopia by Daniel (2008), while the same author reported absence of resistance to norfloxacin, which is similar with the current study's result (Table 1).

Fluoroquinolones (ciprofloxacin and norfloxacin) are the drugs-of-choice to treat the life-threatening salmonellosis (Hopkins et al., 2006; Cui et al., 2009). Particularly in Ethiopia these fluoroquinolones and gentamycin may be the drugs of choice for treating salmonellosis (Ayalu et al., 2011). However, a study carried out in different parts of Ethiopia reported that some *Salmonella* strains were resistant to gentamycin (Samson, 2005; Daniel, 2008;

Table 1. Susceptibility patterns of *Salmonella* species (N = 36) for commonly used antibiotics.

Antibiotic	Susceptibility patterns		
	Resistance No. (%)	Intermediate No. (%)	Sensitive No. (%)
Ampicillin (AMP)	34 (94.4)	1 (2.8)	1 (2.8)
Cefoxitin (FOX)	11 (30.6)	6 (16.7)	19 (52.8)
Cotrimoxazole (SXT)	14 (38.9)	0 (0.0)	22 (61.1)
Chloramphenicol (C)	13 (36.11)	3 (8.3)	20 (55.56)
Ciprofloxacin (CIP)	0 (0.0)	1 (2.8)	35 (97.2)
Norfloxacin (NOR)	0 (0.0)	0 (0.0)	36 (100)
Gentamicin (CN)	0 (0.0)	2 (5.56)	34 (94.44)
Nalidixic acid (NA)	0 (0.0)	5 (13.9)	31 (86.1)
Tetracycline (TE)	19 (52.8)	6 (16.7)	11 (30.6)

P-value < 0.001.

Getenet et al., 2011). The reason for the emergence of resistant *Salmonella* isolates might be due to the use of antibiotics in food animals (WHO, 2001; White et al., 2001). In addition, the extensive use of fluoroquinolones has made fluoroquinolone-resistant *Salmonella enterica* isolates to emerge all over the world (Hopkins et al., 2006; Cui et al., 2009). In general, the present study showed a statistically significant difference between the prevalence of resistant and susceptible *Salmonella* isolates to the tested antibiotics ($p < 0.001$) (Table 1).

Antimicrobial drug resistant *Salmonella* isolates in dairy farm, Bahir Dar, Ethiopia is presented in Table 2. About 34 (94.4%) of the isolated *Salmonella* spp. were resistant to one or more antibiotic agents including the commonly used antimicrobial agents like ampicillin, tetracycline, cotrimoxazole and chloramphenicol. This result is agrees with the result of a study conducted in Tehran, Iran, which showed that 93.9% of *Salmonella* serotypes isolated from meat were found resistant to at least one of the tested nine antibacterial agents (Soltan et al., 2009). Likewise, the current study's result is comparable with the result of a study conducted in Sudan, which showed that 93.1% of *Salmonella* serotypes isolated from humans were found resistant to at least one of the tested nine antibacterial agents (Fadlalla et al., 2012). Similarly, this result is supported by other studies conducted in Ethiopia (Zelalem et al., 2011; Bayeh et al., 2010).

However, in a study conducted in the Black Lion Hospital, Addis Ababa, 6% of *Salmonella* isolates were resilient to at least one of the regularly used antimicrobial agents (Dawit, 1998). And also another study conducted in Jimma Hospital and Jimma Health Center, South West Ethiopia, 54% of *Salmonella* isolates were resistant to at least one of the commonly used antimicrobial agents (Abebe, 2002). This result showed a lower rate of resistant *Salmonella* isolates than the present study result. This might be due to a difference in resistance rate of *Salmonella* isolates from place to place and from time to time. There was no *Salmonella* isolates out of the total 36 *Salmonella* isolates tested were susceptible to all of

the nine tested antibiotics, and ten *Salmonella* isolates were resistant to one tested antibiotic. Ten (27.78%), 4 (11.11%), 11 (30.56%), 1 (2.78%), 0 (0.00%) and 0 (0.00%) of *Salmonella* isolates were resistant to two, three, four, five, six and seven antibiotics out of the nine tested antibiotics, respectively. The high levels of antibiotic resistance might be due to the result of the use of antibiotics in food animals (White et al., 2001).

The incidence of *Salmonella* with multiple resistance food animals can really compromise public health (D'Aoust et al., 1992). It is known that the old and mostly used antimicrobials have multidrug resistance thereby causing public health risks and then encourages using fluoroquinolones and third-generation cephalosporins for empiric treatment (Parry and Threlfall, 2008). *Salmonella* isolates showed relatively little resistance to a number of antimicrobial agents, with 63.89% susceptible to all antimicrobial drugs tested. All isolates were susceptible to norfloxacin followed by ciprofloxacin. During the study period, the *Salmonella* isolates from lactating cows in the dairy farm of Bahir Dar town showed multiple antimicrobial resistance patterns ranging from 2 to 5 antibiotics. Multidrug-resistance pattern of *Salmonella* isolates is shown in Table 3.

In this study, out of the total 36 *Salmonella* isolates, 72.22% (26/336) were multidrug-resistant. Relatively the rate of multidrug-resistant *Salmonella* isolates in the present study was higher than in the study conducted in Ethiopia (Bayleyegn et al., 2003b). This might be due to the probability that multiple antibiotic resistant *Salmonella* spp. increase from time to time (Kariuki et al., 2006) and differ from place to place (Nagal et al., 2006). In line with this, resistance for two or more of antimicrobials which was observed in this study was lower than other study conducted in Addis Ababa, Ethiopia (Zealelem et al., 2011). This could be credited mainly to the unselective use and abuse of antimicrobials both in the farm and human health sectors (Leegaard et al., 1996; Bayleyegn et al., 2003a).

The highest multidrug-resistance was seen against the

Table 2. Antimicrobial drug resistant of *Salmonella* isolates.

Number of drug resisted	Resistant <i>Salmonella</i> isolates	
	Number	Percent
R0	0	0.00
R1	10	27.78
R2	10	27.78
R3	4	11.11
R4	11	30.56
R5	1	2.78
R6	0	0.00
R7	0	0.00

R0 = susceptible to all; R1, R2, R3, R4, R5, R6, and R7, resistant to 1, 2, 3, 4, 5, 6, and 7 antimicrobials tested respectively.

Table 3. Multidrug-resistance pattern of *Salmonella* isolates.

Resistance pattern	<i>Salmonella</i> isolates [No (%)]
Resistance to two antibiotics	
AMP-FOX	5 (13.89)
AMP-TE	1 (2.78)
AMP-C	2 (5.56)
CEF-TE	2 (5.56)
Resistance to three antibiotics	
AMP-FOX-TE	2 (5.56)
AMP- TE-SXT	2 (5.56)
Resistance to four antibiotics	
AMP-FOX-TE-SXT	1 (2.78)
AMP-TE-SXT-C	10 (27.78)
Resistance to five antibiotics	
AMP-FOX-TE-SXT-C	1 (2.78)

AMP: Ampicillin; C: chloramphenicol; (Thrimethoprim-sulfmethoxazole); TE: tetracycline.

FOX: cefoxitin; SXT: cotrimoxazole

commonly used antimicrobial agents, in which resistance to ampicillin tetracycline, cotrimoxazole and chloramphenicol recorded 10 (27.78) followed by resistance to ampicillin and cefoxitin (13.89%). However, antibiotic resistance is increasing to some antibiotics, such as fluoroquinolones and third-generation cephalosporins. These antibiotics are usually used to treat serious infections caused by bacterial pathogens frequently found in food, such as *Salmonella* and *Campylobacter* (Bryan and Doyle, 1995). The use of antimicrobials in food animals has caused the development of antimicrobial resistance (White et al., 2001), through mutation and acquisition of resistance encoding genes (Fluit, 2005).

Resistance for two or more of antimicrobials which was observed in this study did not agree with the study

conducted in Sudan 75% (Fadlalla et al., 2012). As such, due to high resistance of *Salmonella* spp., these commonly used antibiotics mentioned earlier are occasionally used as alternatives (Cui et al., 2009). This augmented the resistance in these antibiotics; the reason is imprudent use of antibiotics by patients and physicians alike in many developing countries such as Ethiopia. This has led to an increased antibiotic resistance and in turn reduced therapeutic efficacy in these countries (Daniel, 2008). In general, *Salmonella* isolates were not resistant to fluoroquinolones, gentamicin and nalidixic acid (Table 1).

Conclusion

High rate of *Salmonella* spp. was isolated from milk of

lactating cows in the study area. And also the study revealed the high rate of drug resistance pattern to commonly used antibiotics among the isolated *Salmonella* spp. In line with this, low level of antimicrobial resistance was observed to fluoroquinolones, gentamicin and nalidixic acid. Based on the findings of this study further detailed work is needed to determine the *Salmonella* spp.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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