



Seroprevalence of *Salmonella* Agglutinins among Apparently Healthy Students of a Tertiary Institution in North-Eastern Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author MYT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OI, ROO and JF corrected the first draft and managed the analyses of the study. Author GAO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the level of *Salmonella* agglutinin and its titre among apparently healthy students of Tertiary Institution in North-eastern Nigeria.

Study Design: A cross-sectional study on Seroprevalence of *Salmonella* agglutinin;

Place and Duration of the Study: Department of Biological Science Technology, Federal Polytechnic Mubi, Adamawa State, between September to December, 2016.

Methodology: This study was a cross-sectional study in which 200 apparently healthy students of Federal Polytechnic Mubi were tested for *Salmonella* agglutinin using both slide and tube agglutination methods.

Statistical Analysis: Non-parametric Mann-Whitney statistics and least significance difference (LSD) were used to test for significant difference in all the data obtained. Significance difference was taken when $P < 0.05$.

Results: The result showed that the number of male with positive Widal agglutinin was significantly higher than that of female ($P=0.028$). The number of positive titre was significantly higher in age group 21-25 years ($P=0.002$) when compared to other age brackets. However, there was no statistical difference between the age bracket 16-20 years and 31-35 years ($P=0.747$). The result of the agglutination titre showed that more male had *Salmonella* titre for *Salmonella* Typhi O (81.8%) and *Salmonella* Typhi H (72.7%) while more female had *Salmonella* agglutinin titre for *Salmonella* Typhi H (44.4%) and *Salmonella* Paratyphi B-H (33.3%). It was also observed that only agglutinin for *Salmonella* Paratyphi A-O, B-O, C-O, B-H and C-H were present in the sera of male subjects up to the titre of 160 and at frequencies ranging from 1.6-58.8%. Furthermore, among females, it was observed that only agglutinins for *Salmonella* Paratyphi A-O, *Salmonella* Typhi C-H and *Salmonella* Typhi O and H were present in the sera of the subjects up to the titre of 160 and at frequencies ranging from 2.5-25.0%.

Conclusion: Detecting *Salmonella* agglutinin titre up to 160 among apparently healthy individuals is of public health significance. Thus, there is need to monitor this trend and create awareness on environmental and behavioural risk factors for *Salmonella* infections.

Keywords: Seroprevalence; *Salmonella*; agglutinins; apparently healthy.

1. INTRODUCTION

Typhoid and paratyphoid fever commonly called 'enteric fever' is caused majorly by *Salmonella* of the serotype Typhi and less commonly the serotype Paratyphi A, B or C. *Salmonella* is a Gram negative facultatively rod-shaped members of the Enterobacteriaceae family. They are non-spore forming with a dimension of 0.7 – 1.5 μm in diameter and 2 – 5 μm in length and are mostly motile using peritrichous flagella [1]. Their complex antigenic structures (such as somatic O antigens, Capsular antigen and Flagella H Antigens) are often used for their classification.

In most developing countries especially in the tropics, typhoid fever is responsible for high rate of morbidity and mortality with an estimated annual global incidence of 21 million cases and more than 700, 000 deaths, while the less severe paratyphoid fever caused an estimated 5.4 million illness in 2000. The morbidity and mortality rate was however reported to be on the increase due to poor sanitation and hygiene, unavailability of vaccines and a high cost of effective antimicrobials [2,3].

Environmental and behavioural risk factors associated with typhoid fever include eating unhygienic food, inadequate washing of hands before eating, drinking contaminated water that was not properly treated and handled, sharing and eating from the same utensils [4].

Typhoid and paratyphoid agents are transmitted majorly by faecal-oral route. The most important

risk factors for the spread of this disease causing agents are apparently healthy carriers, convalescent and chronic carriers which usually shed the bacteria into the environment. Bacteria shed by these carriers contaminate food and water which further aid in the consistent spread of the organisms.

Most health facilities in developing countries like Nigeria relied on Widal test for diagnosing enteric fever [5]. This is because the test is simple, easy, readily available, inexpensive and relatively non-invasive; its diagnostic speed ensures prompt and adequate therapy for the disease [6]. The most prominent drawback of Widal agglutination test is cross-reactions. This occurs when an antibody produced by non-typhoidal antigens reacts with typhoid specific –antigens [7]. Despite the drawback, if properly harnessed and interpreted accurately, Widal test still has significant diagnostic utility [8] in providing presumptive diagnosis for typhoid fever. Therefore, this study was carried out to determine *Salmonella* agglutinin and its titre among apparently healthy students of Federal Polytechnic Mubi.

2. MATERIALS AND METHODS

2.1 Study Area

The study area was Federal Polytechnic Mubi located in Mubi metropolis, Adamawa State, Nigeria. The institution was established by decree no. 33 of 1979 constitution. Mubi

metropolis is a geopolitical area comprising of two local government areas; Mubi North and Mubi South. The metropolis is located between latitudes 10° 05' and 10° 30'N of the equator and between longitude 13° 12' and 13° 19'E of the Greenwich meridian. The two Local government areas occupy a land area of 192,307 Km² and support a total population 260,009 people (National Population Census 2006). The area shares boundary with Maiha L.G.A in the South, Hong L.G.A in the West, Michika L.G.A and Cameroon Republic in the East [9].

2.2 Study Design

This was a cross-sectional study in which 200 healthy students were randomly recruited for the study.

2.3 Sample Collection

Blood samples from 200 apparently healthy students were collected randomly. The blood samples were centrifuged in order to separate the serum from the plasma. The serums collected were immediately used for detection of *Salmonella* agglutinin.

2.4 Widal Agglutination Test

SWE-CARE diagnostic febrile antigen kits (Spain) was used for the purpose of this test. The rapid slide screening test was first carried out followed by the tube agglutination test according to the manufacturer's specifications. The SWE-CARE diagnostic kit is suitable for both rapid slide and tube agglutination test against human sera for the detection of *Salmonella* agglutinins. The stained antigen suspensions are killed bacteria stained to enhance the reading of agglutination test. The blue stained antigens are specific to the somatic 'O' antigens while the red antigens are specific to the flagella 'H'antigens.

Positive and negative control from the kits was used throughout the experiment to authenticate all the results, especially where the agglutination was faint.

2.5 Inclusion and Exclusion Criteria

Participants of this study were drawn from students who are apparently healthy, not on drugs and who have not visited medical facilities three months before consent was sought.

2.6 Statistical Analyses

Non-parametric Mann-Whitney statistics and least significance difference (LSD) were used to test for significant difference in all the data obtained. All statistical analyses were carried out using SPSS version 17. Significance difference was taken when P<0.05.

2.7 Ethical Consideration

Consent of each participant was obtained prior to testing. Ethical approval was also obtained from the institutions' Research and Seminar Committee Board. The individual laboratory results were kept confidential and given to the participants at the completion of the project.

3. RESULTS

From the tested 200 serum sampled, 120 (60.0%) were found to be Widal positive with at least one of the tested antigens (Table 1). Although the results showed that the percentage of Widal positive subjects were not significantly different from Widal negative subject (P=0.221). The result also showed that the number of male with positive Widal agglutinin 81.8% (n=110) was significantly higher than that of female 33.3% (n=30) (P=0.028).

Table 2 showed the prevalence of Widal positive sera in relation to age group of the students. The age of the study population ranged from 16-40 years. The result showed that the age group 21-25 years had the highest (71.3%) Widal positive agglutinin titre, while the lowest agglutinin titre was recorded in the age group 36-40 years. Statistically, the number of positive titre was significantly higher in age group 21-25 years (P=0.002) when compared to other age brackets. However, there was no statistical difference between the age bracket 16-20 years and 31-35 years (P=0.747).

Table 3 showed the distribution of *Salmonella* agglutinin titres in 110 male subjects. The findings showed that more male had *Salmonella* agglutinin titres for *Salmonella* Typhi O (81.8%) and S. Typhi H (72.7%). More so, the most male had *Salmonella* agglutinin titres for S. Paratyphi C-O (56.4%), 50 (45.5%) had agglutinin for S. Paratyphi A-H.

The results in Table 3 also showed the number and percentage of sera with titres in 110 male

students. It was observed that only agglutinins for *S. Paratyphi* A-O, B-O, C-O, B-H and C-H were present in the sera of the subject up to the titre of 160 and at a frequency ranging from 1.6-58.8%. Remarkably, agglutinins for *S. Typhi* O and H were not present in the sera of male students. Also, the percentage of male subjects with titre 1:80 was significantly higher than those with titre 1:160 ($P=0.001$).

Table 4 showed the distribution of *Salmonella* agglutinin titre in 90 female students. The results showed that more female had *Salmonella* agglutinin titres for *S. Typhi* H (44.4%), while the least agglutinin titre for female students was for *S. Typhi* O (7.8%).

The results in Table 4 also showed the number and percentage of sera with end titres in 90 healthy female students. It was observed that only agglutinins for *S. Paratyphi* A-O, C-H and *S. Typhi* O and H were present in the sera of the subjects up to the titre of 160 and at frequencies ranging from 2.5% - 25.0%. Moreover, the

percentage of female subjects with titre 1:160 was significantly lower than those with titre 1:80 ($P=0.001$).

4. DISCUSSION

In this study, 120 (60%) of the 200 (100%) blood samples gave positive Widal reaction. This indicates a high prevalence of typhoid fever in the sampled population. Similarly, a high prevalence of positive Widal reaction greater than 60% was also reported in Nigeria [5,10,11]. The possible explanations for the high prevalence of *Salmonella Typhi* and *Paratyphi* titres among apparently healthy individuals may be that the bacilli are persistent in the human host at sub-clinical levels [12]. It may also be due to the repeated subclinical infections with either of *Escherichia*, *Shigella*, *Citrobacter* or *Proteus* species which shared common 'O' or 'H' antigens with *Salmonella* spp [13,14,15]. Alternatively, other individuals may have developed tolerance to frequent exposure of small inocula of *Salmonella Typhi* and *Paratyphi*

Table 1. Prevalence of Widal positive sera (*Salmonella* agglutinin titre) based on gender

Sex	No. of sera tested (%)	No. of widal positive (%) ^a	No. of widal negative (%) ^a
Male	110 (55.0)	90 (81.8) ^b	20 (18.2)
Female	90 (45.0)	30 (33.3) ^c	60 (66.7)
Total	200 (100)	120 (60.0)	80 (40)

^aa = not significantly different ($P=0.221$). ^bb and ^cc =significantly different ($P=0.028$)

Table 2. Prevalence of Widal positive sera (*Salmonella* agglutinin titre) based of age group

Age group	No. of sample tested	Positive titre (%) ($\geq 1:80$)	Negative titre (%) ($< 1:80$)
16-20	30	16 (53.3)	14 (46.7)
21-25	80	57 (71.3)	23 (28.7)
26-30	56	31 (55.4)	35 (44.6)
31-35	29	14 (48.3)	15 (51.7)
36-40	5	2 (40.0)	3 (60.0)

Table 3. Number (%) and distribution of sera with agglutinin end titre among male students

<i>Salmonella</i> antigen	No. of sera tested	No. (%) of widal positive	Titre < 1:80	Titre 1:80 ^a	Titre 1: 160 ^b
<i>S. Paratyphi</i> A-O	110	31 (28.2)	11 (35.5)	10 (32.3)	10 (32.3)
<i>S. Paratyphi</i> B-O	110	30 (27.3)	4 (13.3)	20 (66.1)	6 (20.0)
<i>S. Paratyphi</i> C-O	110	62 (56.4)	19 (30.6)	40 (64.5)	3 (4.8)
<i>S. Typhi</i> O	110	90 (81.8)	5 (5.5)	85 (94.4)	0
<i>S. Paratyphi</i> A-H	110	50 (45.5)	15 (30.0)	35 (70.0)	0
<i>S. Paratyphi</i> B-H	110	34 (30.9)	6 (17.6)	8 (23.5)	20 (58.8)
<i>S. Paratyphi</i> C-H	110	40 (36.4)	11 (27.5)	29 (72.5)	0
<i>S. Typhi</i> H	110	80 (72.7)	10 (22.5)	70 (87.5)	0

^aa and ^bb = significantly different ($P=0.001$)

Table 4. Number (%) and distribution of sera with agglutinin end titre among female students

Salmonella antigen	No. of sera tested	No. (%) of widal positive	Titre < 1:80	Titre 1:80 ^a	Titre 1: 160 ^b
S. Paratyphi A-O	90	20 (22.2)	5 (25.0)	10 (50.0)	5 (25.0)
S. Paratyphi B-O	90	5 (5.6)	2 (40.0)	3 (60.0)	0
S. Paratyphi C-O	90	6 (6.7)	2 (33.3)	4 (66.7)	0
S. Typhi O	90	7 (7.8)	2 (28.6)	3 (43.0)	2 (28.6)
S. Paratyphi A-H	90	23 (25.6)	6 (26.1)	17 (73.9)	0
S. Paratyphi B-H	90	30 (33.3)	20 (66.7)	10 (33.3)	0
S. Paratyphi C-H	90	29 (32.2)	5 (17.2)	20 (68.9)	4 (13.8)
S. Typhi H	90	40 (44.4)	15 (38.0)	24 (60.0)	1 (2.5)

* a and b = significantly different (P =0.001)

[12] which may lead to a high rate of asymptomatic infections. However, most of the subjects may not be having the active diseases. This was in agreement with the previous observation [16]. Lower prevalence of positive Widal reactions have been reported in Nigeria, other African countries and Asia. These includes 24.4% in Owerri, Nigeria, [17] 41.2% in Borno, Nigeria, [18] 21.3% and 22% prevalence rate in Kenya and Ethiopia respectively, [13,19] 50% prevalence rate in Nepal [8] and 31.1%, 12.1%, 27.3% have been reported in India [20,21,22].

The Widal test reaction involves the use of bacterial suspensions of *S. Typhi* and *S. Paratyphi* A, B and C, treated to retain only the 'O' and 'H' antigens. The antigens suspensions are employed to detect corresponding immunoglobulins in the serum of a patient suspected of having typhoid fever. During the primary immune response in acute typhoid fever, IgM somatic O immunoglobulin appears first, while the IgG flagella H immunoglobulin appear much later, but persists for a longer period in circulation [7,11].

The age distribution pattern of Widal positive test showed that the age group 21-25 years (57 cases) had the highest positive result while the least was found in the age bracket 36-40 years (5 cases). This may be due to the fact that all the subjects in the study area are students of tertiary institution and are within that age population. This result was found to contradict previous findings in which it was reported that 26-35 years and 5-15 years had the highest and lowest Widal positive results respectively [17]. However, the findings of this study were in agreement with previous reports which suggested that the prevalence of typhoid fever in endemic areas was considered high among school-age children and young adults. Older adults are presumably

relatively resistant due to frequent boosting of immunity [23].

Also, in this study, sera from males were more Widal positive than females. This was in agreement with previous studies [11,16,24]. This was probably a reflection of different eating habits and level of personal hygiene [11]. Contrary to our findings, however, higher prevalence rate among female than male was reported [17,20]. According to them, this may be due to the fact that females are more vulnerable to such disease due to poor health conditions and environmental factors associated with these women.

Among the 110 males tested, the titre of *Salmonella* 'O' was higher than those of 'H', whereas in 90 females, *Salmonella* 'H' titres were higher than those of 'O'. This was in agreement with the report of Okonko et al. [11] but differ with the report of Ibekwe et al. [5].

A negative agglutination test may be for one of several reasons which include absence of infection by *S. Typhi*, the carrier state, an inadequate inoculum of bacterial antigen in the host to induce antibody production, technical difficulty or errors in the performance of the test, previous antibiotic treatment and variability in the preparation of commercial antigens.

Apparently healthy refers to the absence of disease based on clinical signs and symptoms normally assessed by routine laboratory procedures and physical examinations. The subjects involved in this study are apparently healthy and are most likely unaware of their health conditions and therefore are likely to infect others. This is because transmission of typhoid fever was reported to be chiefly among apparently healthy carriers especially among those who work as food handlers [25]. Many of

them become asymptomatic carriers after an acute typhoid episode.

The etiologic agent of typhoid can establish a chronic asymptomatic infection of the human gallbladder by forming a biofilm. On reaching the gallbladder, the bacterium can induce active local infection or exist asymptotically in a chronic carrier state [26,27]. The chronic typhoid carrier state can occur following symptomatic or subclinical infections of *S. Typhi*. Among untreated cases, 10% usually shed bacteria for 3 months after the initial onset of symptoms and 2-5% becomes chronic carriers. Bacteria shed by asymptomatic carriers contaminate food and water and account for much of the person-to-person transmission of serovar Typhi in developing countries [26,27,28]. Another study showed that a higher percentage of asymptomatic infection occurs when there is a low dose of the bacterial pathogen [29]. According to them, the low dose of the bacterial pathogen cannot overcome the host immune system to cause active infection or illness.

Typhoid and paratyphoid fevers are common in developing countries, basically due to the problem of unsafe drinking water, inadequate sewage disposal and flooding, [11] poor hygiene practice, indiscriminate and open defecation due to lack of toilet facilities, etc. Health education about personal hygiene, provision of safe water supply, proper sanitation system, excluding disease carriers from food handling are among the critical public health measures that can be taken to prevent typhoid and paratyphoid [11]. Although bacteriological culture remains the gold standard for definitive diagnosis of typhoid fever, lack of its immediate availability during the acute febrile illness may limit its use. Consequently, a rapid, accurate and sensitive test such as the one we have reported may be useful to differentiate typhoidal from non-typhoidal febrile illnesses in a typhoid endemic region.

5. CONCLUSION

The findings of this study established that *Salmonella* agglutinins are common among apparently healthy individuals with variable Widal agglutination titre. Although many studies have highlighted the limitations of Widal test for laboratory diagnosis of typhoid fever, however, the test is still of diagnostic value in endemic areas provided the results are interpreted with an utmost degree of care and accuracy.

CONSENT AND ETHICAL APPROVAL

As per University standard guideline, participant consent and ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ryan KJ, Ray CG. Sherris medical microbiology. 4th ed. McGraw-Hill Companies Inc.; 2008.
2. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Organisation. 2004;82:346-53.
3. Adeshina GO, Osuagwu N, Okeke CE, Ehinmidu JO. Plasmid DNA analysis and conjugative study of antibiotic resistant *Salmonella typhi* and *paratyphi* to ten selected antibiotics in Zaria, Nigeria. Afri. J. Phar. Pharmacol. 2010;4(4):151-157.
4. Ram PK, Naheed A, Brooks WA, Hossain MA, Mintz ED, Breiman RF. Risk factors for typhoid fever in a slum in Dhaka, Bangladesh. Epidemiol. Infect. 2007; 135(3):458-65.
5. Ibekwe AC, Okonko IO, Onunkwo AU, Donbraye E, Babalola ET, Onoja BA. Baseline *Salmonella agglutinin* titres in apparently healthy freshmen in Awka, South Eastern, Nigeria. Scientific Res. Essays. 2008;3(9):225-230.
6. Onunkwo AU, Nwankwo CH, Umolu DN. Stochastic appraisal of the routine serodiagnostic method for enteric fever in Nigeria. J. Sci. Eng. Technol. 2001;8(1): 2964-2973.
7. Olopoenia LA, King AL. Widal agglutination test - 100 years later: Still plagued by controversy. Postgrad. Med. J. 2000; 76(892):80-84.
8. Acharya T, Tiwari BR, Pokhrel BM. Baseline widal agglutination titre in apparently healthy nepalese blood donors. JHAS. 2013;3(1):27-30.
9. Adebayo AA. Mubi Region. A Geographic Synthesis; Paraclete Publishers, Yola. 2004;17-37.
10. Abalaka ME, Osho O, Okolo MO, Adeyemo SO. Prevalence of Typhoid fever among outpatients visiting Ibrahim Badamasi Babangida Specialized Hospital

- and General Hospital in Minna, Nigeria. *J. Biol. Today's World*. 2013;2(3):140-152.
11. Okonko IO, Soley FA, Eyarefe OD, Amusan TA, Abubakar MJ, Adeyi AO, et al. Prevalence of *Salmonella typhi* among patients in Abeokuta, South-Western Nigeria. *Brit. J. Pharmacol. Toxicol.* 2010;1:6-14.
 12. Teddy C, Adias ZA, Jeremiah AO, Ilesanmi. Distribution of antibodies to *Salmonella* in the sera of blood donors in the South-Western Region of Nigeria. *Blood Transfus.* 2010;8:163-169.
 13. Ezzi MS, Anzala O, Maritim MC, Bhatt KM. Distribution of *Salmonella Typhi* antibodies in the sera of healthy blood donors at Kenyatta National Hospital. *Online J. Med. Med. Sci. Res.* 2014;3(3):19-23.
 14. Parry CM, Hoa NT, Diep TS, Wain J, Chinh NT, Vinh H, et al. Value of a single-tube widal test in diagnosis of typhoid fever in Vietnam. *J. Clin. Microbiol.* 1999;37(9): 2882-6.
 15. Colle JG, Marmion FA, Simmons BP. *A Mackie and Mc Cartney Practical Medical Microbiology*. New York: Churchill Livingstone. 1996;389.
 16. Adeleke OE, Adepoju TJ, Ojo DA. Prevalence of typhoid fever and antibiotic susceptibility pattern of its causative agent *Salmonella typhi*. *Nig. J. Microbiol.* 2006;20(3):1191-1197.
 17. Ibegbulam-Njoku Peace N, Chijioke-Osuji CC, Duru FC. Prevalence of antibody titre in healthy individual and enteric fever patients in Owerri, Nigeria. *J. Pub. Health Epidemiol.* 2014;6(6):192-196.
 18. Isa MA, Kubo II, Ismail HY, Allamin IA, Shettima A. Prevalence of *Salmonella* agglutinins among patients attending hospitals in biu, Borno State, Nigeria. *Arch. Appl. Sci. Res.* 2013;5(1):83-87.
 19. Weyesa JB. Seroprevalence of Typhoid fever among subjects with acute febrile manifestations at Tertiary Care Center, Addis Ababa, Ethiopia. *Int. J. Sci. Res.* 2014;3(10):147-155.
 20. Perala BMK, Koripella RL, Cheemala SS. Prevalence of widal positivity in a tertiary care hospital. *Int. J. Res. Rev.* 2016;3(8): 32-35.
 21. Sharma A, Bala K, Agarwal S, Chaudhary U. Seroprevalence of *Salmonella typhi* in a tertiary care facility in North India. *Int. J. Curr. Microbiol. App. Sci.* 2015;4(5):946-949.
 22. Vallab GBB, Vazhavandal G, Uma A, Chitra RP. Prevalence of enteric fever in patients with pyrexia of unknown origin. *Asian J. Biomed. Pharma. Sci.* 2014;4(28): 39-42.
 23. Parry CM. Epidemiological and clinical aspects of human typhoid fever. In *Salmonella Infections: Clinical, Immunological and Molecular Aspects*, ed. Mastroeni P, Maskell D. Cambridge University Press. 2005;1-10.
 24. Esohe AI, Osazua F, Ajayi SA, Ebueku A, Igbiginbe. Dual infection with typhoid and malaria in febrile patients in Ikare Akoko, Nigeria. *Int. J. Trop. Med.* 2012;7(1):49-52.
 25. Sasikumar C, Kannan V, Senthilkumar B. Asymptomatic typhoid carriers in Namakkal District, Tamil Nadu. *J. Environ. Biol.* 2005;26(1):113-115.
 26. Crawford RW, Rosales-Reyes R, Ramirez-Aguilar ML, Chapa-Azuela OC, Alpuche-Aranda C, Gunn JS. Gallstones play a significant role in *Salmonella* spp. gallbladder colonization and carriage. *Proc. Natl. Acad. Sci. of the United States of America.* 2010;107:4353-435.
 27. Gonzaz-Escobedo G, Marshall JM, Gunn JS. Chronic and acute infection of the gall bladder by *Salmonella typhi*: Understanding the carrier state. *Nat. Rev. Microbiol.* 2011;9(1):9-14.
 28. Vandepitte J, Verhaegen J, Engbaek K, Rohner P, Pilot P, Heuck C. Basic laboratory procedures in clinical Bacteriology, 2nd Edn, WHO Geneva; 2003.
 29. Nester DW, Anderson DG, Roberts CE, Pearsall NN, Nester MT. Epidemiology. In: *Microbiology, A Human Perspective*. 2004;491-450.

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