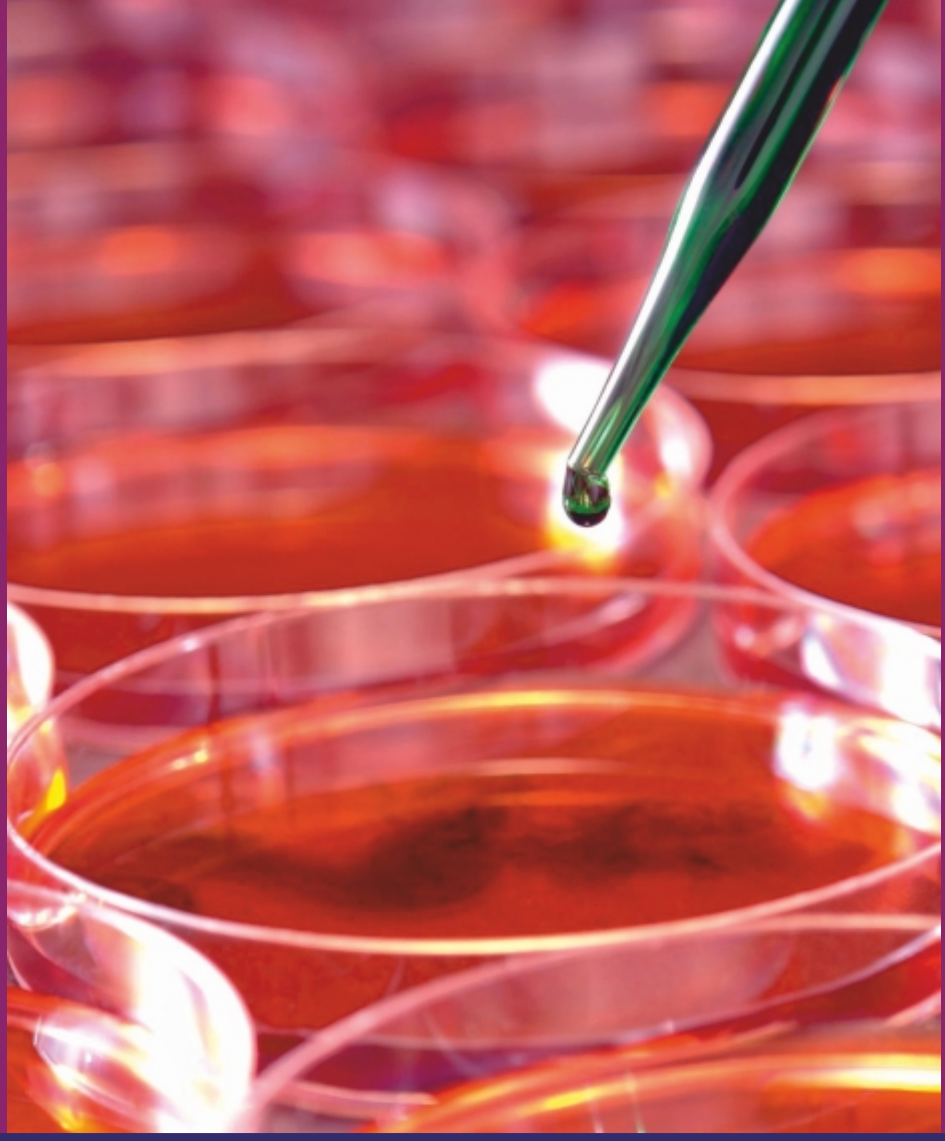
ISSN 1119-0388



Tropical Journal of Medical Research

Official Publication of Medical Research Society, Nnamdi Azikiwe University

Volume 18 / Issue 1 / Jan-Jun 2015

www.tjmrjournal.org

African Index Medicus, AJOL

Review Article

Applications of *Bacillus subtilis* as an important bacterium in medical sciences and human life

Seyed Hossein Shahcheraghi, Jamshid Ayatollahi, Marzieh Lotfi[1]

Department of Infectious Diseases, Infectious and Tropical Diseases Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, [1]Department of Modern Sciences and Technologies, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract

Probiotics are a group of organisms without pathogenic effects. These organisms are known as creatures that have advantageous effects on the safety of their host. The most common kinds of microbes that are used as probiotics are Lactic acid bacteria (LAB) and bifidobacteria; but several yeasts and bacteria may also be used. In this study, we have reviewed the benefits of *Bacillus subtilis* as a probiotic bacterium. The journals published from 1989 to 2012, have been used in the study. At the start of the twentieth century, these organisms were thought to usefully affect their host by ameliorating the intestinal bacterial equilibrium, and therefore, injuncting the toxin-producing organisms and pathogens. Nowadays, particular health effects are being examined and documented, including the prohibition and remedy of pathogen-induced diarrhea, palliation of severe intestinal inflammatory diseases, urogenital infections, and atopic cases. Probiotics are generally used as a division of foods with particularly added active live cultures, including soy yogurt, yogurt or as dietary supplements. *B. subtilis* strains produce antibiotics and enzymes that are important in both medical and industrial sciences.

Keywords: *Bacillus subtilis*, microbes, probiotics

Introduction

The word *probiotic* is related to live organisms that are creatures that are beneficial to their host.[1-3]

Bacteria that are probiotic have the numerous applications. This is because of a range of feasible health effects of probiotics. Probiotics are available in the form of powders, capsules, enriched yogurts, yogurt-like products, and milk. The health effects related to them include, cholesterol-lowering, immune system excitation, and prohibition of cancer aggression.[1,3-7]

Probiotics, in recent reviews,[8-11] have been introduced as agents that are useful in the prohibition of necrotizing enterocolitis in premature children. Due to the enthusiasm during conduction of revoews, precaution is recommended by the Scientific Foundation, as short- and long-term safety have not yet been established.[12,13]

Access this article online	
Quick Response Code:	Website: www.tjmrjournal.org
	DOI: 10.4103/1119-0388.152530

The explanation of accessible data on probiotic organisms is further separated by dose, variation of variant selection, delivery vehicle, and assessment of livability and impression.[14]

The lactic acid produced by LAB and their natural conformity to the gut condition has provided them the benefit of being used as probiotics, more than other organisms.[15] Most studies have also been restricted to *Bacillus*.[16,17]

The basic interest in the *Bacillus* species, as probiotic organisms, has only come about since the last 15 years.[18-20]

The *Bacillus* species that have been most comprehensively investigated are *Bacillus subtilis, Bacillus clausii, Bacillus cereus, Bacillus coagulans,* and *Bacillus licheniformis*.[21,22]

Bacillus subtilis, as a probiotic organism, has beneficial effects on both animals and humans.[23,24]

The primary aim of this review is to investigate the benefits of *B. subtilis* in medical and industrial sciences.

Address for correspondence: Dr. Seyed Hossein Shahcheraghi, Infectious and Tropical Diseases Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
E-mail: shahcheraghih@gmail.com

The journals published from 1989 to 2012 have been used in the study.

Features of *Bacillus subtilis*

B. subtilis is a catalase-positive bacterium that is also known as the 'hay *bacillus*' or 'grass *bacillus*'.

This bacterium is an endospore-forming, aerobic, rod-shaped organism popularly found in soil, water, and as an aggregation in plants. *B. subtilis* and its close *Bacilli* are the main source of industrial enzymes such as proteases and amylases. It has also been used for investigating protein secretion and for expansion as a host for the generation of inharmonious proteins.[25]

Under conditions of nutritional esurience, *B. subtilis* stops growing and begins its responses to restitute growth by enhancement of the metabolic activity. These responses include the implantation of motility and chemotaxis and generation of antibiotics and macromolecular hydrolases (proteases and carbohydrases).[26]

Bacillus subtilis directly secretes proteins into the culture medium, shortening the filtration of the recombinant proteins. This bacterium does not produce endotoxins.[27]

Several vectors have been recognized for the production process of proteins in *B. subtilis*.[28-30]

The high expression of human cytokines is still an existing problem in *B. subtilis*. In addition, few studies on the production in this organism have been reported.[31-34]

B. subtilis is a very good model for discussion on peptidoglycan.[35]

B. subtilis has the ability to take up and recombine extracellular DNA into its genome, which makes it naturally qualified for genetical transformation.[36]

The genome sequence related to *B. subtilis* has supplied a great understanding about the lifestyle of the bacterium. This bacterium is not a pathogen. Interestingly, the components of this genome have encoded many pathways for the use of plant molecules.[37]

The growth of biofilms is perceived when *B. subtilis* is inseminated on the roots of *Arabidopsis thaliana*.[38,39]

B. subtilis is an important probiotic bacterium. Therefore, we reviewed the advantages of *Bacillus subtilis* as a probiotic bacterium.

Applications of *Bacillus subtilis*

Lactobacillus, *Saccharomyces*, *Streptococcus*, *Aspergillus spp.,* and *Bacillus* are the important probiotics.[40]

They have beneficial effects on their host, which depends on their potency to endure oxygen stressors, heat, and osmotic stress during storage and processing.[41]

A strain related to *B. subtilis* (LS 1-2) can ameliorate intestinal bacterial balance and the gut health of broilers. Also, it can be used as a growth promoter in diets related to broilers.[42]

Bacillus subtilis is a bacterium that has been successfully planned to express inharmonious antigenic factors genetically fused to proteins of the spore coat, as a vaccine vehicle endowed with considerable resistance against heat, and affects as a probiotic organism for both animals and humans.[43]

B. subtilis strains are also used in the generation of bacterial vaccines either as antigen bearers or as antigen cellular manufactories. The application of *B. subtilis* spores as vaccine vehicles, delivered through mouth, has attracted noticeable attention because of its probiotic effects and the extended shelf-life.[44-46]

In another study, it was demonstrated that *B. subtilis* (strain NC11) is a potential probiotic, and displays a good role as an inhibitor against infection related to *Salmonella enteritidis* in the intestinal epithelial cells.[47]

Another study has shown that *B. Subtilis (WD161)* — producer of amylase — can be applied to co-culturing with *Clostridium butylicum (TISTR 1032)*. This will increase the production of acetone–butanol–ethanol (ABE) from starch without an anaerobic remedy.[48]

The *B. subtilis* strain, AS-S01a, is a bacterium that produces a high rate of alkaline alpha-amylase. It may be applied as a good bacterial source in the production of a high rate of alkaline alpha-amylase under an appropriate situation. This enzyme does not need the Ca2+ ion for performing its action as a detergent and it shows consistency in the presence of enzymes, such as, protease, surfactant, oxidant, and metal ion chelating agents.[49]

Bacillus subtilis (natto) Takahashi, as a natto starter, is generally applied for producing a fermented product, which has been a traditional food in Japan for more than a 1000 years.[50] It also produces milk-clotting enzymes.[51]

Fibrinolytic enzymes in the bacteria have a dissolvent role about fibrin clots and are evidently used as thrombolytic

agents.[52] These enzymes have been detected in various bacteria, the most important among which is the genus *Bacillus,* specially *Bacillus subtilis,* from traditional fermented foods.[53,54] The bacterial numbers, water quality, and growth during early development of white shrimp enhances the effect of the *Bacillus subtilis* in the shrimp.[55]

In another study, *Bacillus subtilis E20,* a probiotic bacterium, was demonstrated to have the potency to increase growth in white shrimp by enhancing the action of the digestive enzymes and food attraction.[56]

Aerobic fermentation is a common method for producing enzymes in *B. subtilis* strains (e.g. amylase, protease), insecticides, antibiotics, purine nucleotides, polyglutamic acid, D-ribose, polyhydroxybutyrate (PHB), and so on. These products are important in both medical and industrial sciences.[57-62]

Acute and subchronic toxicity testing have now been widely examined on *B. subtilis* var. *natto* in *in vitro* studies. These studies are conducted on animals. Their results protect the application of *B. subtilis* as a food supplement.[63]

Summary

This review shows that *B. subtilis* is an important probiotic bacterium. The application of *Bacillus subtilis* as a probiotic is increasing quickly with the enhancing number of studies demonstrating immune stimulation, antimicrobial activities, and competitive exclusion. The single and most important benefit of products related to this bacterium is that they can be produced easily and the stability of the final product can be assured. Furthermore, they can be incorporated into everyday foods.

References

1. Fuller R. Probiotic foods. Current use and future developments. Int Food Ingred 1993;3:23-6.
2. Fuller R. Probiotics in man and animals. J Appl Bacteriol 1989;66:365-78.
3. Lee YK, Salminen S. The coming of age of probiotics. Trends Food Sci Technol 1995;6:241-5.
4. Sanders ME. Summary of conclusions from a consensus panel of experts on health attributes of lactic cultures: Significance to fluid milk products containing cultures. J Dairy Sci 1993;76:1819-28.
5. Salminen S, Isolauri E, Salminen E. Clinical uses of probiotics for stabilizing the gut mucosal barrier: Successful strains and future challenges. Antonie Van Leeuwenhoek 1996;70:347-58.
6. de Simone C, Rosati E, Moretti S, Bianchi-Salvadori B, Vesely R, Jirillo E. Probiotics and stimulation of the immune response. Eur J Clin Nutr 1991;45:32-4.
7. Elmer GW, Surawicz CM, McFarland LV. Biotherapeutic agents. A neglected modality for the treatment and prevention of selected intestinal and vaginal infections. JAMA 1996;275:870-6.
8. AlFaleh K, Anabrees J. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev 2014;4:CD005496.
9. Barclay AR, Stenson B, Simpson JH, Weaver LT, Wilson DC. Probiotics for necrotizing enterocolitis: A systematic review. J Pediatr Gastroenterol Nutr 2007;45:569-76.
10. Deshpande G, Rao S, Patole S. Probiotics for prevention of necrotising enterocolitis in preterm neonates with very low birthweight: A systematic review of randomised controlled trials. Lancet 2007;369:1614-20.
11. Martin CR, Walker WA. Probiotics: Role in pathophysiology and prevention in necrotizing enterocolitis. Semin Perinatol 2008;32:127-37.
12. Boyle RJ, Robins-Browne RM, Tang ML. Probiotic use in clinical practice: What are the risks? Am J Clin Nutr 2006;83:1256-64; quiz 1446-7.
13. Neu J. Perinatal and neonatal manipulation of the intestinal microbiome: A note of caution. Nutr Rev 2007;65:282-5.
14. Shanahan F. Probiotics: A perspective on problems and pitfalls. Scand J Gastroenterol Suppl 2003;34-6.
15. Guerra NP, Bernárdez PF, Méndez J, Cachaldora P, Castro LP. Production of four potentially probiotic lactic acid bacteria and their evaluation as feed additives for weaned piglets. Anim Feed Sci Technol 2007;134:89-107.
16. Kumprecht I, Zobac P. Continuous application of probiotics based on Saccharomyces- cerevisaevar elipsoideus and *Bacillus* CIP-5832 in the nutrition of chicken broilers. Zivocisna Vyroba 1996;41:311-6.
17. Casula G, Cutting SM. *Bacillus* probiotics: Spore germination in the gastrointestinal tract. Appl Environ Microbiol 2002;68:2344-52.
18. Hong HA, Duc le H, Cutting SM. The use of bacterial spore formers as probiotics. FEMS Microbiol Rev 2005;29:813-35.
19. Mazza P. The use of *Bacillus subtilis* as an antidiarrhoeal microorganism. Boll Chim Farm 1994;133:3-18.
20. Sanders ME, Morelli L, Tompkins TA. Sporeformers as human probiotics: *Bacillus*, *Sporolactobacillus*, and *Brevibacillus*. Compr Rev Food Sci Food Saf 2003;2:101-10.
21. Barbosa TM, Serra CR, La Ragione RM, Woodward MJ, Henriques AO. Screening for *Bacillus* isolates in the broiler gastrointestinal tract. Appl Environ Microbiol 2005;71:968-78.
22. Spinosa MR, Braccini T, Ricca E, De Felice M, Morelli L, Pozzi G, *et al*. On the fate of ingested *Bacillus* spores. Res Microbiol 2000;151:361-8.
23. Paccez JD, Luiz WB, Sbrogio-Almeida ME, Ferreira RC, Schumann W, Ferreira LC. Stable episomal expression system under control of a stress inducible promoter enhances the immunogenicity of *Bacillus subtilis* as a vector for antigen delivery. Vaccine 2006;24:2935-43.
24. Shahcheraghi SH, Nowruzi J, Javadi G, Shahhoseini MH, Samadi H. Frequency of pXO1 gene in *Bacillus cereus, Bacillus thuringiensis* and *Bacillus subtilis* by SDS-PAGE Technique. J Sabzevar Univ Med Sci 2010;17:196-206.
25. Shahcheraghi SH, Nowruzi J, Shahhoseini MH, Javadi G, Moradi H. Investigation of pXO plasmid frequency in non-pathogenic *Bacilluses* by observating plasmid and protein bands in electrophoresis. J Microbiol Biotech 2010;2:41-8.
26. Villéger R, Saad N, Grenier K, Falourd X, Foucat L, Urdaci MC, *et al*. Characterization of lipoteichoic acid structures from three

probiotic Bacillus strains: Involvement of D-alanine in their biological activity. Antonie Van Leeuwenhoek 2014;106:693-706.
27. Harwood CR. *Bacillus subtilis* and its relatives: Molecular biological and industrial workhorses. Trends Biotechnol 1992;10:247-56.
28. Stragier P, Losick R. Molecular genetics of sporulation in *Bacillus subtilis*. Annu Rev Genet 1996;30:297-341.
29. Simonen M, Palva I. Protein secretion in *Bacillus* species. Microbiol Rev 1993;57:109-37.
30. Ozdamar TH, Sentürk B, Yilmaz OD, Calik G, Celik E, Calik P. Expression system for recombinant human growth hormone production from *Bacillus subtilis*. Biotechnol Prog 2009;25:75-84.
31. Lin HH, Yin LJ, Jiang ST. Expression and purification of *Pseudomonas aeruginosa* keratinase in *Bacillus subtilis DB104* expression system. J Agric Food Chem 2009;57:7779-84.
32. Liu Y, Lu F, Chen G, Snyder CL, Sun J, Li Y, *et al*. High-level expression, purification and characterization of a recombinant medium-temperature alpha-amylase from *Bacillus subtilis*. Biotechnol Lett 2010;32:119-24.
33. Lam KH, Chow KC, Wong WK. Construction of an efficient *Bacillus subtilis* system for extracellular production of heterologous proteins. J Biotechnol 1998;63:167-77.
34. Min-Young S, Cheol-Ho P, Sangkee R, Su-Il K. Design of an expression vector and its application to heterologous protein expression in *Bacillus subtilis*. Biotechnol Lett 2002;24:1869-74.
35. Westers L, Dijkstra DS, Westers H, van Dijl JM, Quax WJ. Secretion of functional human interleukin-3 from *Bacillus subtilis*. J Biotechnol 2006;123:211-24.
36. Westers L, Westers H, Quax WJ. *Bacillus subtilis* as cell factory for pharmaceutical proteins: A biotechnological approach to optimize the host organism. Biochim Biophys Acta 2004;1694:299-310.
37. Foster SJ. The role and regulation of cell wall structural dynamics during differentiation of endospore-forming bacteria. Soc Appl Bacteriol Symp Ser 1994;23:25-39S.
38. Chen I, Dubnau D. DNA uptake during bacterial transformation. Nat Rev Microbiol 2004;2:241-9.
39. Kunst F, Ogasawara N, Moszer I, Albertini AM, Alloni G, Azevedo V, *et al.* The complete genome sequence of the gram-positive bacterium *Bacillus subtilis*. Nature 1997;390:249-56.
40. Bais HP, Fall R, Vivanco JM. Biocontrol of *Bacillus subtilis* against infection of Arabidopsis roots by *Pseudomonas syringae* is facilitated by biofilm formation and surfactin production. Plant Physiol 2004;134:307-19.
41. Rudrappa T, Quinn WJ, Stanley-Wall NR, Bais HP. A degradation product of the salicylic acid pathway triggers oxidative stress resulting in down-regulation of *Bacillus subtilis* biofilm formation on Arabidopsis thaliana roots. Planta 2007;226:283-97.
42. Tannock GW. Molecular assessment of intestinal microflora. Am J Clin Nutr 2001;73:410-4S.
43. Ross RP, Desmond C, Fitzgerald GF, Stanton C. Overcoming the technological hurdles in the development of probiotic foods. J Appl Microbiol 2005;98:1410-7.
44. Sen S, Ingale SL, Kim YW, Kim JS, Kim KH, Lohakare JD, *et al.* Effect of supplementation of *Bacillus subtilis LS 1-2* to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology. Res Vet Sci 2012;93:264-8.
45. Deshpande G, Rao S, Patole S. Progress in the field of probiotics: Year 2011. Curr Opin Gastroenterol 2011;27:13-8.
46. Ferreira LC, Ferreira RC, Schumann W. *Bacillus subtilis* as a tool for vaccine development: From antigen factories to delivery vectors. An Acad Bras Cienc 2005;77:113-24.
47. Ricca E, Cutting SM. Emerging applications of bacterial spores in nanobiotechnology. J Nanobiotechnology 2003;1:6.
48. Oggioni MR, Ciabattini A, Cuppone AM, Pozzi G. *Bacillus* spores for vaccine delivery. Vaccine 2003;21 Suppl 2:S96-101.
49. Thirabunyanon M, Thongwittaya N. Protection activity of a novel probiotic strain of *Bacillus subtilis* against *Salmonella enteritidis* infection. Res Vet Sci 2012;93:74-81.
50. My Tran HT, Cheirsilp B, Hodgson B, Umsakul K. Potential use of *Bacillus subtilis* in a co-culture with *Clostridium butylicum* for acetone–butanol–ethanol production from cassava starch. Biochem Eng J 2010;48:260-7.
51. Roy JK, Rai SK, Mukherjee AK. Characterization and application of a detergent-stable alkaline α-amylase from *Bacillus subtilis* strain AS-S01a. Int J Biol Macromol 2012;50:219-29.
52. Shih IL, Yu YT. Simultaneous and selective production of levan and poly (gamma-glutamic acid) by *Bacillus subtilis*. Biotechnol Lett 2005;27:103-6.
53. Shieh C-J, Phan Thi L-A, Shih I-L. Milk-clotting enzymes produced by culture of *Bacillus subtilis* natto. Biochem Eng J 2009;43:85-91.
54. Peng Y, Yang X, Zhang Y. Microbial fibrinolytic enzymes: An overview of source, production, properties, and thrombolytic activity *in vivo*. Appl Microbiol Biotechnol 2005;69:126-32.
55. Batomunkueva BP, Egorov NS. Isolation, purification, and resolution of the extracellular proteinase complex of *Aspergillus ochraceus* 513 with fibrinolytic and anticoagulant activities. Microbiology 2001;70:519-22.
56. Tough J. Thrombolytic therapy in acute myocardial infarction. Nurs Stand 2005;19:55-64; quiz 66.
57. Nimrat S, Suksawat S, Boonthai T, Vuthiphandchai V. Potential *Bacillus* probiotics enhance bacterial numbers, water quality and growth during early development of white shrimp (Litopenaeus vannamei). Vet Microbiol 2012;159:443-50.
58. Liu KF, Chiu CH, Shiu YL, Cheng W, Liu CH. Effects of the probiotic, *Bacillus subtilis* E20, on the survival, development, stress tolerance, and immune status of white shrimp, *Litopenaeus vannamei* larvae. Fish Shellfish Immunol 2010;28:837-44.
59. Schallmey M, Singh A, Ward OP. Developments in the use of *Bacillus* species for industrial production. Can J Microbiol 2004;50:1-17.
60. Shi S, Chen T, Zhang Z, Chen X, Zhao X. Transcriptome analysis guided metabolic engineering of *Bacillus subtilis* for riboflavin production. Metab Eng 2009;11:243-52.
61. Tännler S, Zamboni N, Kiraly C, Aymerich S, Sauer U. Screening of *Bacillus subtilis* transposon mutants with altered riboflavin production. Metab Eng 2008;10:216-26.
62. Zhang X-Z, Zhang Y-H. One-step production of biocommodities from lignocellulosic biomass by recombinant cellulolytic *Bacillus subtilis*: Opportunities and challenges. Eng Life Sci 2010;10:398-406.
63. Hong HA, Huang JM, Khaneja R, Hiep LV, Urdaci MC, Cutting SM. The safety of *Bacillus subtilis* and *Bacillus indicus* as food probiotics. J Appl Microbiol 2008;105:510-20.

How to cite this article: Shahcheraghi SH, Ayatollahi J, Lotfi M. Applications of *Bacillus subtilis* as an important bacterium in medical sciences and human life. Trop J Med Res 2015;18:1-4.

Source of Support: Nil, **Conflict of Interest:** None declared.