Journal of Applied & Environmental Microbiology, 2020, Vol. 8, No. 1, 1-5
Available online at http://pubs.sciepub.com/jaem/8/1/1
Published by Science and Education Publishing
DOI:10.12691/jaem-8-1-1

What is the Origin of Human Bacterial Flora?

Alen J Salerian MD*

40 Nestoros street ,Vavrona, 19016 Greece
*Corresponding author: alensalerian@gmail.com

Received December 15, 2019; Revised January 30, 2020; Accepted February 18, 2020

Abstract The number of microorganisms that inhabit human body- normal human flora- is estimated to be 38 trillion and equal to the number of human cells. Historically , the human flora have been viewed as friendly immigrant microbes that enhance immunity against pathogens and in general promote better health for humans. This reviewinvestigates the origin of human flora for it may have important implications to combat opportunistic infections. Of importance, the breast milk and tissue bacteria have been demonstrated not to be contaminants from skin. The unique composition of the bacterial communities in breast milk being different from bacteria from other body parts ,makes it less likely that they were translocated from gut or oral cavity, suggesting either bacteremia or transformation from organic matter as the possible pathways of origin. The documented endogenous origin of the bacteria in breast milk and tissue, the presence of microorganisms in several anatomically well insulated human organs along with the data consistent with the possible endogenous origin of Malassezia and H.Pylori , two species of the normal human flora. suggest at least some of the 38 trillion bacteria inhabiting the human body may not be foreign immigrants .The origin of normal human floraand the precise mechanisms and pathways of origin remains unknown yet the evidence is consistent with an endogenous origin., Further experimental validation of these observations are necessary. A more complete understanding of the human flora may be of help for intelligentstrategies for preventing, diagnosing and treating opportunistic infections.

Keywords: *human flora, bacteremia, endogenous infections, malassezia, h.pylori, breast milk bacteria*

Cite This Article: Alen J Salerian MD, "What is the Origin of Human Bacterial Flora?." *Journal of Applied & Environmental Microbiology*, vol. 8, no. 1 (2020): 1-5. doi: 10.12691/jaem-8-1-1.

1. What is the Origin of Human Bacterial Flora?

The number of microorganisms that inhabit the human body-the normal human flora- is estimated to be 38 trillion and equal to the number of human cells [1].

Historically, the human flora has been viewed as friendly immigrants that enhance immunity against pathogens and in general promote better health for humans. The functions of the normal flora include digestion of substrates, production of vitamins, stimulation of cell maturation, stimulation of the immune system, aid in intestinal transit and colonization resistance.

In the last decade diverse molecular observations have revealed the existence of endogenous microorganisms in several body parts which had previously been thought to be sterile. For instanceit has been shown that breast milk [2,3] breast tissue, [4], endometrium [5], uterus [6] amniotic fluid, [6,7,8] placenta, [9,10,11] umbilical cord blood [12], meconium [13,14] lungs [15] and semen [16,17] and bladder [18,19,20,21,22] harbor bacterial communities. Of significance, the composition of the bacterial communities is unique for each habitat suggesting that they may have different origins [23].

Surprisingly, these molecular discoveries consistent with the possible existence of endogenous bacterial communities in human organs have not altered the medical paradigm that presumes the human flora resultsfrom contamination by foreign microorganisms.

The aim of this review is to address four questions:

A. Are all or some of the 38 trillion bacteria of human flora Immigrants?
B. How can we explain the presence of endogenous microbes inplacenta ,,umbilical cord blood, breast tissue, breast milk and amniotic fluid?
C. What is the origin of Mallasezia and H.Pylori species that belong to normal flora and have coevolved with humans?
D. Does the human tissues contain the essentials to produce microorganisms and has organic matter ever produced microorganisms?

The answers to the above questions may have important implications for human health for theymay introduce novel strategies to combat diverse opportunistic infections.

I will first review the breast milk microbiome that has been proven not to be contaminants from skin ,discuss the anatomical and physical barriers in some organs that seem to be incompatible with invasion by foreign microorganisms, review Malassezia and H. pylori species which seem to share the signature traits of endogenous species and finally address the implications of these findings.

2. The Breast Milk and Tissue Microbiome

Recent molecular studies showed that the breast milk bacteria are not contaminants from skin [3,4]. Additionally, molecular studies of the breast tissue bacteria revealed that Proteobacteria is the most abundant phylum in breast tissue, unlike in the vagina, oral cavity, bladder, skin, and gastrointestinal tract [2]. This finding suggests that breast tissue may have a unique microbiota, distinct from that found at other body sites.

Animal studies in mice suggested transport of bacteria in maternal blood to the mammary glands involving gut dendritic cells and macrophages [24]. It has also been presumed that gut dendritic cells maytransport bacterial components to the lactating breast in humans [25,26]. However, thishypotheses seems to be inconsistent with the molecular evidence suggesting morphological dissimilarities between gut and breast tissue bacteria.

Also, the source of bacteria in the breast tissue has been proposed to be bacteria in oral cavity through bacteremia. The distinct compositions of the bacterial communities in the breast tissue and oral cavity make this hypotheses less likely. Still, bacteremia from oral cavity is a possible pathway however it is not a common occurrence among healthy subjects as indicated by a study which showed it occurred in approximately 5.5 cases per million population per annum [27].

Another possibility may be of an endogenous origin. Thishypotheses is consistent with the history of life on earth and the observation that human tissues have the essentials to produce bacteria [28,29] and supported by the presence of bacteria in the blood of healthy subjects confirmed by two independent studies [30,31].

3. Possible Origin of Diverse Bacterial Communities in Humans

Novel findings in microbiology suggest fetus is not sterile placenta [10,11,12], amniotic fluid [7,8,9], breast milk [2,3], umbilical cord blood [13] are frequently colonized with bacteria,. Diverse hypotheses have been proposed to explain the presence of bacterial communities in healthy fetus yet it has been firmly established that the historical paradigm of a sterile fetus is incorrect.The predominant proposals about the origin of commensal bacteria in healthy fetus are the following.: Contamination from skin, bacteremia and translocation.

In general microorganisms are passive travelers highly sensitive to environmental influences as they greatly depend on the flow of air, water , body fluids etc. to contaminate new hosts. Certain physical and anatomical barriers make it almost impossible for microorganisms to penetrate and travel through healthy human tissues without access to the blood stream or lymphatic system.

It has been demonstrated that bacteria are quite often present in the placenta: of healthy subjects. in 27% of 195 investigated placentas, intracellular bacteria could be morphologically demonstrated in the placental basal plate [10].

Aagaard and colleagues determined the microbiome of preterm and full−term placentas and identified a unique bacterial community [11]. The detected bacterial DNA was mostly from nonpathogenic commensals belonging to phyla Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes and Fusobacteria. When comparing this placental microbiome with that of other body sites, it most closely resembled the microbiome of the oral cavity [11]. Dissimilarity to the microbiome of stool or the vagina made it unlikely that the placental bacteria had been the result of contamination. It has been hypothesized that bacteremia from the oral bacteria maybe the source of the of bacteria In the placenta, yet this seems unlikely considering the lack of any evidence of illness or sepsis in healthy pregnant women.Also, the documented rarity of bacteremia from the oral cavity suggests the proposed oral -placental transmission via bacteremia is less likely.In addition, the well publicized case report of oral-placental transmission of infection via bacteremia indicated serious infection resulting in stillbirth and cannot be generalized to healthy pregnancies [32].

How do we know whether uterus is or is not involved in the bacterial communities in the placenta?

The idea that healthy uterine cavity is sterile has been challenged yet It is unknown whether the bacteria present in the uterus during pregnancy are natives or invaders. To study microbiome composition and its characteristics in the womb of pregnant women, 41 decidual tissue and 64 amniotic fluid samples were investigated by Zhu and colleagues [6] They concluded that uterus contained microbiome with low diversity and bacterial colonization did occur during healthy pregnancy. They also observed that the microbiome structure of amniotic fluid was more diverse that of decidual tissue, which supported the previous reports that bacteria could be hematogenously spread from blood to amniotic cavity as suggested by Aagaard et al. [6,11].

Moore and colleagues tested the hypothesis that the uterus of virgin heifers and pregnant cows possessed a resident microbiome by 16S rRNA gene sequencing of the virgin and pregnant bovine uterus. They concluded that the uterine microbiome is established by the time a female reaches reproductive maturity, and that pregnancies are established and maintained in the presence of a uterine microbiome [33].

Traditionally , an abnormal endometrial microbiota has been associated with implantation failure, pregnancy loss, and other gynecological and obstetrical conditions. [5] Recent molecular studies detected low biomass microbiomes in endometrium previously considered sterile [5].

Noteworthy is the observation that the presence of bacterial communities in uterus and endometrium raises more questions about the origin of bacteria in placenta and amniotic for the composition of bacterial communities in these tissues seem to be different than the normal flora of vagina. This finding indirectly supports the possibility that their origin is either through transmission via blood and bacteremia or some other yet unidentified pathway and hence consistent with an endogenous origin.

The presence of bacteria in healthy female bladder documented by molecular studies may also deserve some attention for the higher internal pressure of bladder versus external air pressure would make the upstream travel of microbes in healthy subjects nearly impossible [18,19,20,21,22]. However their origin remains unknown.

In essence the observations summarized above suggest multiple human tissues harbor bacteria which are not contaminants yet their precise origin remains unknown. Among many hypotheses of origin, bacteremia and transformation of human tissues to microorganisms seem to be possible yet they both lack experimental validation.

From a broader perspective of life in universe , certain similarities between the human flora and bacteria in extraordinarily remote locations on earth. For instanceAndean lakes 4400 m above sea level,,completelyisolated, exposed to extreme environmental factors are the habitat of enormous populations of bacteria resistant to antibiotic [34]. Where do these species come from? Are they immigrants? Or is it possible that they are living witnesses of transformation of living things from nonliving organic matter?

Also, Riding and Thomas observed that bacterial colonies in the decaying organic matrix had formed peloidal crusts from Early Cretaceous reef carbonates in eastern Spain [35].

Worthy of emphasis is “the decaying organic matter as the origin of bacteria and peloidal crusts.

4. Malassezia and H.Pylori Species

A gradually evolving paradigm suggests microorganisms do not always harm the fetus and this observation may also be true for humans for often the host biology seems to play a predominant influence in whether bacteria act as pathogens or friendly coexisting species like Malassezia and H.pylori species.

Among countless microorganisms that belong to the normal human flora,, Malassezia and H.pylori species. seem to distinguish themselves as possible candidates of endogenous origin: they have coevolved with humans dating back to East Africa 56,000 years ago and their distribution around the world seems to be mediated by pathways independent of contamination and more specifically it does not correspond to host to host transmission [36,37,38] In essence, clinical, epidemiological and molecular evidence suggests they are not imported contaminants and yet under certain conditions - which are almost always associated with the alteration of host biology-they may become disease inducing pathogens.

Malassezia species are not contagious,and host to host transmission does not seem to be the predominant pathway for infections [36]. Experimental studies indicate that the inoculation of Malassezia species does not cause infections without occlusion which means altering host skin temperature and humidity [39].

Collectively these observations suggest infections and Malassezia species may develop without contamination or contaminants.

Malassezia yeasts have coevolved with humans dating back to east Africa 56.000 years ago show global distribution mimicking human migration and display adaptable, biological properties related to their human host [40]. Also,there is no difference in antigen titers between healthy people with normal flora and people with tinea versicolor [41]. These observations suggest Malassezia species are a part of normal human physiology.

Malassezia species seem to be governed by host biology as Cushing disease , intake of nonsteroidal anti-inflammatory agents may produce infections [42]. The opposite is also true as the elimination of these influences eradicate infections [42]. These observations are consistent with the governing influence of host biology in Tinea Versicolor infections.

Host genetics seem to play an important role in the development of tinea versicolor infections [43].

H. pylori species are a part of normal human physiology and flora [37,38]. Human biology seems to govern H. Pylori species: Cushing syndrome [43], Cushing ulcers [44], Curling ulcers [45,46] and Zollinger Ellison disease [47] ,histamine injections [48], NSAIDs [49] induce peptic ulcers. These observations suggest host biology governs H. Pylori infections associated with peptic ulcers.

Molecular evidence also support the endogenous origin of H. pylori species:Pathways independent of contamination -in contrast to host to host transmission- seem to be the predominant influence in the prevalence of Infection; with the geographical distance from East Africa corresponding to human migration and micro evolution of greater H. pylori virulence [37,38]. In addition host genetics independent of the immunocompetence mediate H. pylori species consistent with the possibility of their endogenous origin [50].

In summary diverse and converging evidence supports the possibility I’m an endogenous origin of Malassezia and H. pylori species or we can say that it is impossible to rule out the possibility of their endogenous origin.

5. Discussion

Traditionally, the ubiquity of bacteria has made it possible to attribute all infections to contamination by dismissing any other pathway that may lead to bacterial growth and infections. Noteworthy is the reality that, organic matter is as ubiquitous as bacteria and thus any discussion about the origin of infections and microorganisms must consider both bacteria and organic matter.

Taken individually, each piece of evidence consistent with the hypotheses that the human bacterial flora may be endogenous may seem inadequate, yet collectively in the complexity of human biology it seems reasonable to consider the possibility of an endogenous human flora. It is also true that it is impossible to rule out the possibility that bacteremia from the oral cavity or the gastrointestinal tract may be the central pathway of origin of the human flora.

Interestingly, the demonstration that bacteremia may occur in healthy subjects seem to support the two leading hypotheses of origin of human flora: endogenous versus gut or oral bacteria originated bacteremia.

Of importance, the breast milk and tissue bacteria have been demonstrated not to be contaminants from skin. Furthermore, the molecular evidence consistent with the unique composition of the bacterial communities in breast milk being different from bacteria from other body parts , argues against the proposed translocation hypotheses.

Collectively, the documented endogenous origin of the bacteria in breast milk and tissue, along with the presence of microorganisms in several anatomically well insulated human organs suggest at least some of the 38 trillion bacteria inhabiting the human body may not be foreign immigrants although the precise mechanisms and pathways of their origin remainunknown.

In addition,the epidemiological clinical and molecular data seem to support the great likelihood of Malassezia and H.Pylori -two common species of the normal human flora- being endogenous.

Further experimental validation of these observations are necessary.

If indeed the origin of some of the bacterial flora is endogenous, it is reasonable to consider the possibility that some opportunities infections result from human tissues.A more complete understanding of the human flora may be of help for intelligent strategies for preventing, diagnosing and treating opportunistic infections.

References

[1] Sender R, Fuchs S, Milo R, 2016. Revised Estimates for the Number of Human and Bacteria Cells in the Body. /journal. pbio. 1002533.

[2] Urbaniaak,C.et al. (2014) Microbiota in human breast tissue. Appl.Environ. Microbiology 80, 3007 - 3014.

[3] Cabrera-Rubio R, Collado MC, Laitenen K, Salminen S. ,Isolauri E,,Mira A, 2012. The human milk microbiome changes over lactation and is shaped by maternal weight and mode of delivery. The American Journal of clinical nutrition, volume 96, issue 3, Page is 544 - 551.

[4] Martin R, Langa S, Reviriego C, et al 2003, Human milk is a source of lactic acid bacteria for the infant gut☆The Journal of Pediatrics. Volume 143, Issue 6, December 2003, Pages 754-758

[5] Moreno E, Franasiak JM, (2017). Endometrial microbiota-new player in town, Fertility and Sterility,Volume 108, Issue 1, Pages 32-39,

[6] Zhu L,Luo F,Hu W et al. (2018) Bacterial Communities in the Womb During Healthy Pregnancy. Front. Microbiol.,

[7] Markenson GR,Adams LA,Hoffman DE,Reece MT, (2003). Prevalence of Mycoplasma bacteria in amniotic fluid at the time of genetic amniocentesis using the polymerase chain reaction. The Journal of Reproductive Medicine, 01 Oct 2003, 48(10): 775-779

[8] DiGiulio DB, (2012), Diversity of microbes in amniotic fluid, Seminars in Fetal and Neonatal Medicine,Volume 17, Issue 1,Pages 2-11.

[9] Collado, M., Rautava, S., Aakko, J. *et al.* Human gut colonisation may be initiated *in utero* by distinct microbial communities in the placenta and amniotic fluid. *Sci Rep* **6,** 23129 (2016)

[10] Stout MJ,, Conlon B, Landeau M, Lee I, Bower C, Zhao Q, Roehl KA, Nelson DM,. Macones GA, I Mysorekar IU, (2008) Identification of intracellular bacteria in the basal plate of the human placenta in term and preterm gestations,American Journal of Obstetrics and Gynecology,Volume 208, Issue 3

[11] Aagaard K, Ma J,Antony KM, Ganu R, Petrosino J, Versalovic J, (2014). The Placenta Harbors a Unique Microbiome ,Science Translational Medicine 21 May 2014: Vol. 6, Issue 237, pp. 237ra65

[12] Jimenez E, Fernandez L, Marin ML, et al, 2005, Isolation of Commensal Bacteria from Umbilical Cord Blood of Healthy Neonates Born by Cesarean Section. Current Microbiology , Volume 51, Issue 4, pp 270-274.

[13] Jimenez E., Marin M.L., Matin R., Odriozola J., M.Olivares M., Xaus J., Fernandez L.,, Rodriguez J.M., Is fetal meconium sterile? Research in Microbiology. (2008). 159, 3, 187-189.

[14] Ardissone A.N., DeLa Cruz D., Davis- Richardson A. G., Rechigi K.T.,et al, Meconium microbiome analysis identifies bacteria correlated with premature birth. PLOS ONE 9(6): e 10 1399.

[15] Beck JM, Young VB, Huffnagle GB, 2012, The microbiome of the lung. Translational Research. Volume 160, Issue 4, October 2012, Pages 258-266

[16] Fourie J, Loskutoff N, Huyser C, 2012, Elimination of bacteria from human semen during sperm preparation using density gradient centrifugation with a novel tube insert, Andrologia, Volume44, Issues 1 Pages 513-517.

[17] Weng S L, Chiu C M, Bacterial communities in semen from men of infertile couples; sequencing reveals and relationships up seminal microbiota to semen quality. Plos one; 9(10): e 110152.

[18] Wolfe, A. J. et al. Evidence of uncultivated bacteria in the adult female bladder. J. Clin. Microbiol. 50, 1376-1383 (2012).

[19] Pearce, M. M. et al. The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. MBio 5, e01283-14 (2014).

[20] Hilt, E. E. et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. J. Clin. Microbiol. 52, 871-876 (2014).

[21] Thomas-White, K., Brady, M., Wolfe, A. J. & Mueller, E. R. The bladder is not sterile: History and current discoveries on the urinary microbiome. Curr. Bladder Dysfunct. Rep. 11, 18-24 (2016).

[22] Whiteside, S. A., Razvi, H., Dave, S., Reid, G. & Burton, J. P. The microbiome of the urinary tract-a role beyond infection. Nat. Rev. Urol. 12, 81-90 (2015).

[23] Costello EK, Lauber CL, Hamady M, Fierer N, Gordon JI, Knight R 2009. Bacterial community variation in human body habitats across space and time. *Science* **326***:* 1694-1697.

[24] Donnet-Hughes, A., Perez, P., Doré, J., Leclerc, M., Levenez, F., Benyacoub, J., . . . Schiffrin, E. (2010). Potential role of the intestinal microbiota of the mother in neonatal immune education. Proceedings of the Nutrition Society, 69(3), 407-415.

[25] Fernández L, Langa S, Martín V, Maldonado A, Jiménez E, Martín R, Rodríguez JM, (2013). The human milk microbiota: Origin and potential roles in health and disease,Pharmacological Research, Volume 69, Issue 1, Pages 1-10.

[26] Rodríguez, JM, 2014, The Origin of Human Milk Bacteria: Is There a Bacterial Entero-Mammary Pathway during Late Pregnancy and Lactation?, Advances in Nutrition, Volume 5, Issue 6, Pages 779-784.

[27] Afra, K., Laupland, K., Leal, J. *et al.* Incidence, risk factors, and outcomes of Fusobacterium species bacteremia. *BMC Infect Dis* **13,** 264 (2013).

[28] Cavalier-Smith T., Cell evolution and Earth history :Stasis and revolution. Philosophical transactions Royal Society in London biological sciences. 2006 June 29;; three six one(1470) 969 - 1006.

[29] Salerian AJ , 2017,Human body may produce bacteria,Medical Hypotheses, 103: 131-132.

[30] McLaughlin RW, Vali H,, Lau PCJ,, Palfree RGE,, De Ciccio A, Sirois M, Ahmad D, Villemur R, Desrosiers M, Chan ECS, (2002) Are There Naturally Occurring Pleomorphic Bacteria in the Blood of Healthy Humans? Journal of Clinical Microbiology 40 (12) 4771-4775

[31] Nikkari S, McLaughlinIJ, Bi W,Dodge DE, Relman DA, (2001), Does Blood of Healthy Subjects Contain Bacterial Ribosomal DNA? Journal of Clinical Microbiology May 2001, 39 (5) 1956-1959;

[32] Fardini Y, Chung P, Dumm R, Joshi N, Han YW, (2010). Transmission of Diverse Oral Bacteria to Murine Placenta: Evidence for the Oral Microbiome as a Potential Source of Intrauterine Infection .Infection and Immunity Mar 2010, 78 (4) 1789-1796.

[33] Moore SG, Erickson AC, Poock SE, Melendez P, Lucy MC, (2017). Hot topic: 16S rRNA gene sequencing reveals the microbiome of the virgin and pregnant bovine uterus. Journal of Dairy Science,Volume 100, Issue 6, Pages 4953-4960.

[34] Dib JR, Weiss A, NeumannA, Ordonez O, Esatevez MC, Farias ME, (2009) Isolation of Bacteria from Remote High Altitude Andean Lakes Able to Grow in the Presence of Antibiotics. Recent Patents on Anti-Infective Drug Discovery, Volume 4, Number 1, 2009, pp. 66-76(11).

[35] Riding R, Thomas S, (2005) Stromatolite reef crusts, Early Cretaceous, Spain: bacterial origin of in situ-precipitated peloid microspar? Sedimentology 365-3091

[36] He S.M., Du W.D., Yang S., Zhou S.M., Li W., Wang J., Xiao F.L., Xu S.X., Zhang X.J., The genetic epidemiology of tinea versicolor in China. Mycosis/ volume 51/ issue one. October 2007.

[37] Suerbaum S, Josenhans (2007) C, Helicobacter pylori evolution and phenotypic diversification in a changing host. Nature Reviews Microbiology volume5, pages441-452 .

[38] **Antonello Covacci1, A ,Telford J L, Giudice G D, Parsonet J, Raappuoli R, 1999,** Helicobacter pylori Virulence and Genetic Geography, *Science* Vol. 284, Issue 5418, pp. 1328-1333

[39] Faergemann J., Fredricksson T., Experimental infections in rabbits and humans with Pityrosprum orbiculare and P.ovale. Journal of Investigative Dermatology. Volume ssue 3, September 1981, Pages 314 - 318.

[40] Faergemann J., Antibodies to Pityrosporum orbiculare in patients with tinea versicolor and controls of various ages , Journal of Investigative Dermatology, 1983 Feb; 80(2): 133-5.

[41] Gaitanis G, Valegraki A, Alexopoulos EC, Kapsanaki Gotsi et. Al. (2009) E,Malassezia furfur fingerprints as possible markers for human phylogeography. The ISME Journal (2009) 3, 498-502.

[42] Burke, R., Tinea Versicolor: susceptibility factors in experimental infection in human beings. Journal of investigative dermatology. 1961, volume 36 number 5, Pages 389 - 401.

[43] Nieman, LK; Ilias, I (December 2005). "Evaluation and treatment of Cushing's syndrome". The American Journal of Medicine. 118 (12): 1340-6.

[44] Pruitt, Basil A. Jr.; F.D. Foley & John A. Moncrief (October 1970). "Curling's ulcer: a clinical-pathology study of 323 cases". Annals of Surgery. 172 (4): 5

[45] Biteghi-bi-Nzeng A, Wang Y, 2008, Cushing's ulcer in traumatic brain injury. Chinese Journal of traumatology (English edition) Volume 11, issue 2, Pages 114-119.

[46] Moody, F. G.; Cheung, L. Y. (Dec 1976). "Stress ulcers: their pathogenesis, diagnosis, and treatment". The Surgical Clinics of North America. 56 (6): 1469-1478.

[47] Meko, M.D, J. B.; Norton, M.D, J. A. (1995-02). "MANAGEMENT OF PATIENTS WITH ZOLLINGER-ELLISON SYNDROME". Annual Review of Medicine. 46 (1): 395-411

[48] HAY, L. J. ; VARCO, R. L. ; CODE, C. F. ; WANGENSTEEN, O. H.1942, The experimental production of gastric and duodenal ulcers in laboratory animals by the intramuscular Injection of histamine in beeswax.: Surgery, Gynecology and Obstetrics Vol.75 pp.170-182.

[49] Huang J-Q., Sridhar S., Hunt R.,2002, Role of helicobacteri infection and none steroidal anti-inflammatory drugs in peptic ulcer disease; and meta-analysis. The Lancet.,, Volume 359 issue 9300, Pages 14 - 22

[50] Malaty HM, Engstrand L, Pedersen NL, Graham DY. *Helicobacter pylori* Infection: Genetic and Environmental Influences: A Study of Twins. Ann Intern Med. ; 120: 982-986.