Antibiotics, the microbiome, and immunity: a case study in Bangladesh

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ANTIBIOTICS, THE MICROBIOME, AND IMMUNITY: A CASE STUDY IN BANGLADESH

By

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ABSTRACT

The purpose of this study was to establish a theoretical impact of antibiotic misuse on the microbiome and its effects on immunity. Recent studies indicate the importance of a microbiome on the immune system. Both the leptin signaling pathways and CD4T cell production are influenced by the microbiome (Mazmanian, et al. 2005). The use of antibiotics has been associated with dysbiosis, a microbial imbalance in the digestive tract. The immune system is limited when the microbiome is dysbiotic.

A case study in Bangladesh is used to show the possible impact of antibiotics on immunity. The indigenous population had lower rates of treatment failure in the treatment of malaria, as compared to the non-indigenous populations. The information known about the microbiome in Bangladesh and the cultural use of antibiotics allow for the theoretical principles to be applied to this case study. The treatment failure of the non-indigenous population may be due to the use of antibiotics that creates dysbiosis in turn limiting immunity.
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Introduction

The microbiome is influential in the health outcomes of an individual. A variation in the microbiome of two populations leads to different health outcomes. Misuse of antibiotics leads to dysbiosis of the microbiome impacting health and immunity. In the presented case study treatment failure for malaria is higher in the non-indigenous populations that misuse antibiotics. The indigenous populations, which have a cultural use of botanicals, had lower rates of treatment failure. It stands to reason that the misuse of antibiotics, which leads to dysbiosis, has the potential to create treatment failure when treating malaria.

Figure 1 Theoretical association between antibiotic misuse and treatment failure.

Problems arise when the microbiome becomes disrupted (dysbiosis), which influences health and impacts homeostatic systems. Lack of bacteria in the digestive tract has been shown to reduce the vascularization of the intestines (Umeskai, 1984), and disrupt the synthesis of essential vitamins (Allen and Stabler, 2008). In addition to function and nutrient synthesis, the microbiome has a direct impact on immunity through leptin signaling pathways and CD4 T cell production (Mazmanian, et al. 2005). Dysbiosis, which disrupts these pathways, has been shown to reduce vaccine efficacy (WHO, 2009), further impacting the health outcomes of an individual.
One way in which the microbiome reaches dysbiosis is by antibiotic use. Antibiotics can decimate the healthy bacteria residing in the intestinal tract. One time antibiotic use requires 4 weeks to 6 months recovery of the microbiome (Phillips 2009). There is a correlation between antibiotics use and the impairment of immune responses within the cells (Abt et al., 2012). Antibiotic use not only influences immune system pathways, but also allows for the establishment of pathogenic bacteria colonies (Looft, et al. 2012).

A variation of the microbiome may occur within two populations, if each population experiences different early life influences. For example, a population that has the cultural tradition of antibiotic use, will experience a disruption in the microbiome. The use of antibiotics will shift the microbiome from beneficial bacteria to pathogenic bacteria. This continual disruption in the microbiome leads to a diminished immune response. Recovery from diseases is then prolonged from the reduced immune function. If the second population favors alternative therapies, the deleterious effects of antibiotics on the microbiome are limited, allowing for a better health outcomes. The difference in the therapy seeking behaviors of the two populations can create a difference in immunity.

In Bangladesh, there is a difference in recovery of malaria between two different populations with similar geographic locality and lifestyles. Before the release of the artemether and lumefantrine malaria medication, a larger percent of the indigenous population made a full recovery while being treated with chloroquine (CQ) and primaquine (PQ). The non-indigenous populations from the
same area had higher rates of treatment failure (Kawai, et al. 2011). The contrasting treatment failure has been studied and there has been no significant understanding of this pre-existing difference (Kawai, et al. 2011). One possible explanation of the difference in recovery is that the populations have varying microbiomes influencing immunity and treatment failure.

Antibiotic use and prescription of antibiotics are unregulated in Bangladesh (Islam 2006). Most non-indigenous persons purchase antibiotics in inadequate doses (Ahmed, Tomson, et al. 2005) and without consulting a physician (Istúriz and Carbon 2000). When consulting a physician, misdiagnosis and prescriptions for antibiotics are prevalent (Islam 2008). While non-indigenous populations utilize antibiotic therapies, indigenous populations rely heavily on alternative therapies such as herbal medicine (Kadir, Sayeed and Mia 2012).

Analysis of the case study in Bangladesh can be further expanded to include the effects of global antibiotic misuse. Complications with antibiotic use arise from inappropriate prescription and patient compliance. Studies surrounding the long term health and immunological effects of antibiotics on the microbiome are still in their infancy. In theory inappropriate use of antibiotics and the resulting dysbiosis can negatively impact immunity enough to cause treatment failure.

**The Microbiome**

The microbiome is the genetic makeup of the bacteria that occupies the human body. Although the microbiome isn’t inherited genetically it is
transferred from mother to offspring via parturition and nursing. This paper will focus primarily on the intestinal microbiome, as recent studies have shown a correlation between the intestinal microbiome and health outcomes. The microbiome can be disrupted by the introduction of antibiotics, leading to growth of pathogenic microbes in the intestinal tract and potential health concerns. An unhealthy or deficient microbiome has been linked with decreased vaccine efficacy in developing countries, and potentially treatment failure.

Digestive microorganisms are made up of two different types of bacteria. The first, Bacteroidetes, is often referred to as beneficial bacteria as it is non-pathogenic and aids the body in digestion and immune support. The second type of bacteria found in the gut is Proteobacteria. Proteobacteria consists of pathogenic bacteria such as E. coli and Salmonella. Low doses of antibiotics can disrupt the microbiome and increase the percentage of Proteobacteria in the gut (Looft, et al. 2012). Lacking beneficial bacteria and having greater percentages of pathogenic bacteria cause immunological changes. After disruption from such occurrences, the microbiome can take years to rebound (Phillips 2009).

The human digestive tract is inhabited by $10^{13}$-$10^{14}$ microorganisms. That is 10 times the eukaryote cells that make up the human body (Gilbert and Epel 2009). These microorganisms are composed of Bacteroidetes and Proteobacteria (Monira, et al. 2011).

To remain in the gut, both Bacteroidetes and Proteobacteria form a biofilm, a mucosal layer that allows the bacteria to adhere to the intestines
(Sonnenburg, Angenent and Gordon 2004). There are different microbial variations in different regions of the intestines. The variations in intestinal cell composition and function allow for a variation in the bacteria colonizing the digestive track (Gilbert and Epel 2009).

Products of microbial synthesis aid the body in several processes as well as induce gene expression in the cells (Gilbert and Epel 2009). Without bacteria present the circulatory system and function of the intestines are stunted. The villi of the cell fail to form complete vascular structures (Umeskai 1984). Germ Free mice (with no gut bacteria) have very little vascularization as compared to conventionally raised mice. Gnotobiotic mice (mice inoculated with bacteria) showed an increase in vascularization within 10 days independent of age (Stappenbeck, Hooper and Gordon 2002). Along with vascularization, the microbiome has an intricate and complex interaction with the immune system.

Inheritance of Microbiome

The critical time period for the prevention of malnutrition and growth faltering is within the first two years (Victoria, 2011). Before a child reaches toddler age, the microbiome is unstable and chaotic. At this age the microbiome is influenced by birthing method, breastfeeding, and the influence of nutrition, infection, and antibiotic use (Albenberg, 2014).

The microbiome is thought to be spread from mother to child during delivery and breastfeeding. Babies that are born via c-section have altered microbiome compositions, often lacking the microbiome diversity of vaginally
born children. The sterility of the c-section surgery prevents that child from gaining exposure to a plethora of microbes to which the vaginally born child is exposed (Ley, Peterson and Gordon 2006). If a child is breastfed, they are exposed to many benefits of breast milk. One such benefit of breast milk is the human milk oligosaccharides (MOS). These MOS are bioactive compounds that remain indigestible throughout the intestinal tract; instead MOS have been shown to nourish specific bacterial colonies (Ley, Peterson and Gordon 2006). Breastfeeding is positively correlated with the beneficial bacteria Bifidobacterium. Bifidobacteria reduce intestinal endotoxin levels (Griffiths, 2004), strengthen the intestinal mucosal layer (Wang, 2006), and has been associated with immune system development and maturation (Hart, 2004), benefiting later health outcomes (Stark, 1982).

Further evidence shows the significance of early life influence on the outcome of the microbiome. Related mice have more similar microbiome colonization than non-related mice (Ley, 2006). This animal model has been tested with humans and resulted in similar outcomes. Human siblings have been shown to have a more similar microbiome as compared to their significant other (Ley, Peterson and Gordon 2006). This evidence indicates early life environment as being a crucial factor in microbiome development.

Understanding how the microbiome is obtained and developed is important to understanding later life health outcomes. Populations that have higher rates of C-sections will have a weakened microbiome, while populations who have higher rates of breastfeeding will have a strengthened microbiome.
These early life influences have a lifelong impact on an individual. As the microbiome is closely associated with immunity, a weakened microbiome could have deleterious health outcomes.

**Microbiome and Health**

The microbiome is an inherent part of health and immunity in an individual. The microbes in the microbiome can synthesize several different vitamins and micronutrients important to immune function. The microbiome has a role in the synthesis of vitamins B12, B6, A, and essential enzymes for immunity. In addition to aiding immunity through synthesis, the microbiome is also responsible for direct interaction with immune cells promoting a holistic immunity through immune response.

The synthesis of vitamins is important in the function of a healthy immune system. The synthesized nutrients of the microbiome include Vitamin A and the active form of Vitamin B6. These are both vitamins that cannot be synthesized by humans (Allen and Stabler 2008). Deficiency of B6 is linked to a decrease in cell-mediated immune responses as well as impaired antibody production (Rail, 1996).

Correlations between the microbiome and Vitamin A have already been established. A disruption of the microbiome can create a disruption of Vitamin A and affect intestinal homeostasis (Cha, et al. 2010). In addition to the maintenance of intestinal homeostasis, Vitamin A stimulates immunity and
reduces morbidity from disease (Cassani, et al. 2012). Vitamin synthesis and absorption is influenced by the microbiome. A disruption or diminishment in the microbiome can create deleterious immune and health outcomes.

As well as vitamin synthesis, non-pathogenic intestinal bacteria (Bacteroidetes) synthesize necessary enzymes for host immune response. The microbiome synthesizes short-chain fatty acids (SCFAs) through fermentation (Maslowski, et al. 2009). The continual presence of SCFAs aids symbiosis of non-pathogenic intestinal bacteria. A diminishment of SCFAs can lead to dysbiosis, a shift in microorganisms from Bacteroidetes to a more pathogenic Proteobacteria. The production of Acetate, a specific SCFA, decreases the prevalence of pathogenic E. coli in the intestines and helps to establish and maintain the healthy gut barrier function (Fukuda, et al. 2011). A lack of homeostasis or disruption of beneficial intestinal bacteria decreases SCFAs promoting enteric disease burden from pathogenic microbes.

Intestinal bacteria promote a healthy immune function through several direct pathways. The microbiome interacts directly with immune cells with Leptin signaling and through lymphoid tissue in the gut. The interaction between bacteria and lymph tissues in the gut results in B-lymphoid expansion (Rhee, et al. 2004) and T cell response (Lord, 1998).

Response of T-helper 1 cells and the inhibition of the T regulatory cells from proliferation are the result of Leptin signaling (Lord, et al. 1998). The hormone Leptin is responsible for the activation and promotion of innate immune
cells. Decreased cellular immunity may be due to low levels of Leptin (Kau, et al. 2011). In germ free mice, which lack a microbiome, there is an increased requirement for Leptin signaling and a lack of CD4 T cell population (Mazmanian, et al. 2005). Bacteroidetes, non-pathogenic bacteria, express polysaccharide-A (PSA) in the presence of B. fragilis. The expression of PSA regulates immune response via expansion of the T helper cells. It suppresses the inflammation caused by enteric pathogens (Mazmanian, et al. 2005). The microbiome aids in the regulation and promotion of cellular immunity.

Multiple interactions of the microbiome with the immune system promote and strengthen immunity. The microbiome synthesizes vitamins and enzymes inherent in immune function. This synthesis not only aids immune function but also acts as a limiting agent on pathogenic intestinal bacteria. Direct interaction between the microbiome and the immune system results in greater CD4T production and a suppressed inflammatory response. A healthy microbiome will aid immune function and health.

**Disruption of the Microbiome**

The health of the microbiome is important to immune function and health. Disruption and dysbiosis of the microbiome has a deleterious effect on the immune system. Enteric pathogens are increased in the microbiome from inappropriate antibiotic use, leading to decreased cellular response to infections.

One time antibiotic use creates a decrease in Bacteroidetes allowing
pathogenic bacteria to form a foothold. After the disruption caused by one time antibiotic use there is a 4 week to 6 month recovery period for the reestablishment of commensal bacteria. This process could take as long as 2 years before a full recovery is made (Phillips 2009).

One time antibiotic use causes a temporary disruption of the microbiome, while continual inappropriate subtherapeutic doses of antibiotics increase the proportion of pathogenic bacteria in the intestines. A low dose feed through antibiotic is common in livestock production, creating a great opportunity for studying the effects of long-term subtherapeutic doses of antibiotics on the microbiome.

In an animal model of swine, 2 groups of swine were fed the same diet. The control group received nothing else in supplementation. The experimental group received sub-therapeutic doses of antibiotics in their feed. The three antibiotics that are fed consisted of chlortetracycline, sulfamethazine, and penicillin. After only three weeks the microbiome consisted of 11% Proteobacteria in the experimental group, while the control group maintained a 1% Proteobacteria composition. Antibiotic resistance was seen in the gut flora long after antibiotic pressures had stopped. Of the Proteobacteria in the experimental group 62% was E. coli. Long-term exposure to subclinical amount of antibiotics can reduce diversity and shift populations of intestinal bacteria to the pathogenic Proteobacteria (Looft, et al. 2012).

The period of dysbiosis and reduced bacteroidetes limits the beneficial
effects of the microbiome on the immune system. Reduction of the gut microbiome from broad-spectrum antibiotic use results in blunted B and T cell response, as well as decreased cell response to other infections (Ichinohe et al., 2011). After an exposure to lymphocytic choriomeningitis virus (LCMV) and mucosal influenza, the use of antibiotics impaired antiviral responses (Abt et al., 2012).

The microbiome can become disrupted from short and long term antibiotic use. This dysbiosis impacts the immunity by limiting immune response. Antibiotic use has been linked with impaired cellular response in cases of infection.

**Microbiome and Vaccine Efficacy**

Immune function and the microbiome are closely connected. Disruption of the microbiome from antibiotic use impairs the immune system. Dybiosis and enteric disease burden negatively influences both immunity and vaccine efficacy.

Vaccine efficacy is impacted by the presence of pathogenic intestinal microbes. In several studies children with a higher enteric disease burden showed a decreased vaccine efficacy as compared to healthy children. In a study from 2009, children from a westernized industrialized country showed a 95% vaccine efficacy rate to the rotavirus vaccine, whereas children from an unindustrialized country (Malawi) showed a lower response rate of 49% (WHO, 2009). Children from the non-industrialized country with higher rates of enteric
disease burden demonstrated lower oral polio vaccine efficacy (Grassly, et al. 2009).

The microbiome’s effects on the production and response of CD4 T cells are important in vaccine efficacy. Vaccines create proper antibody response by utilizing CD4 T cell response to pathogens (Pashine, Valiante and Ulmer 2005). Dysbiosis disrupts the microbiome influencing the Leptin signaling pathways, which maintains levels of CD4 T cells. Pathogenic bacteria establishment in the intestines limits immune response and decreases vaccine efficacy.

In summary, the microbiome of the gut is very important to immune function and response. Direct interaction of the microbiome with the immune systems promotes CD4 T cell production and promotes healthy cellular response to infection. A disruption of the microbiome is costly to health. The use of antibiotics as well as the presence of enteric disease can blunt immune response and vaccine efficacy.

Case Study

The practical application of the microbiome and immunity complex can be applied to a case study occurring in Bangladesh. This case study will illuminate the potential for antibiotics to interfere with clinical recovery of patients diagnosed with P. falciparum, through the disruption of the microbiome. This will be further be debated by looking at the cultural differences between the populations that would promote the theory of antibiotics influence on the treatment failure of
malaria.

**Background**

In 2004, there was a noted difference in the percentage of treatment failures between the indigenous and non-indigenous populations (Kawai, et al. 2011). This difference was first noted by nurses, and later confirmed to be true. A study examining the difference in treatment failure took place at the Christian Hospital Changreafhona, Kaptai Thana, Rangamati district, an area with a mixed population of indigenous and non-indigenous people, from July to September. Patients who were older than 6 months presenting with uncomplicated *P. falciparum* malaria were included in this study. All patients were outpatients and returned on days 1, 2, 3, 7, 14, 28, and any day that they had a fever. All patients were treated with a combination therapy of chloroquine (CQ) and primaquine (PQ). Treatment efficacy was classified by WHO guidelines as adequate clinical and parasitological response (no fever or presence of parasites on days 4-14), early treatment failure (severe malaria, fever, and increased parasitism on days 0-3), late clinical failure (severe malaria, fever, and parasitemia after day 3) and late parasitological failure (presence of parasites after day 14, with no fever). Of the 45 patients, 30 members were of minority-indigenous groups. 19 patients had adequate clinical and parasitological response; early treatment failure, 16; late clinical failure, 8; and late parasitological failure, 2. Of the indigenous patients approximately 53% had an
adequate clinical and parasitic response, while only 14% of the non-indigenous population had ACPR, independent of antimalarial resistant malaria (Kawai, et al. 2011).

There could be several reasons for this difference in treatment failure. This paper will outline the potential difference between the populations, ultimately looking at the microbiome. Both the indigenous and non-indigenous populations reside in the same area in this study. Age as an influence on immunity is a potential cause of treatment failure, but this study showed no correlation between age and treatment failure. The difference in selection effects from early life deaths between the indigenous and non-indigenous populations is inconclusive. There are no known anti-parasitic linked genetic differences between the two populations. Difference in antibiotic use and potential microbiome difference of the two populations will aid the understanding of the deviation in treatment failure between the two populations.

**Pharmacodynamics and Pharmacokinetics**

The microbiome would be unaffected by the antimalarials as these drugs target only the malaria parasite. Antimalarials, although classified as an antimicrobial, do not affect the microbiome. Instead antimalarials target malaria parasites specifically. Knowledge of treatment failure cannot be fully realized until the pharmacodynamics and pharmacokinetics of antimalarials are understood.

For the Kawai Study, Chloroquine (CQ) and Primaquine (PQ) are used in
combination. CQ interferes with the detoxification of the falciparum inhibiting its growth. Resistant parasites accumulate less CQ, decreasing their sensitivity to the drug and allowing them to survive in higher drug concentrations. PQ has a schizonticidal activity, the drug is most effective against gametocytes by blocking oxidative metabolism (Bray, et al. 2005).

In conclusion, the use of antimalarials would not alone create the division in treatment failure of the two populations. Antimalarials have no effect on the bacteria of the microbiome, targeting only the malarial parasite.

**Natural Immunity to Malaria**

The indigenous population may have a superior natural immunity to malaria through prior exposures or through a genetic mutation that protect against malaria. As the results of the Kawai study were independent of age, immunity though prior exposures is unlikely. There is no current evidence to support anti-parasitic linked genetic differences or selection effects in the populations. The difference of treatment failure likely lies with a different cause.

Age is a factor that affects immunity from repeated exposures. For persons living in an endemic area, immunity to malaria can be developed after repeated exposure. According to Giha (2010), the most reliable marker for immunity is age. Immunity through age is associated with pf332-c231 antigen in humans. Antibodies gained from previous malaria infections are effective against several serotypes that can occur during reinfection (Giha, et al. 2010). After the age of twenty malaria infections become less prevalent. Individuals over the age
of fifteen can have asymptomatic infections, in which no fever is present, from retained immunity through repeated exposures (Augustine, et al. 2009). The age sample of the case study was 12.2 ±11.0 years, no correlation between age and treatment failure was noted (Kawai, et al. 2011). In this case study, age has no direct impact on the differences between treatment failures.

The indigenous population could have higher rates of treatment success from early life selection pressures. Placental malarial presence and infant mortality rates influence selection pressures. There is no available data on the infant mortality rate differences between the indigenous and non-indigenous populations, but the Rangamati district has the lowest infant mortality rate of Bangladesh (BBS, 2009).

Placental malarial presence has the potential to create selection pressures via transplacentally acquired antibodies. During pregnancy, malaria can cross the placenta and cause oxygen depreciation and abortion of the fetus. If a mother has malaria at the time of birth, the transplacentally acquired antibodies of a newborn offer no significant protection from malaria in early childhood. Therefore there is no correlation of levels of placental antibodies and age of malarial onset (Achidi, 1996). The long term effects of malaria infection at birth are still unknown (Desai, 2007). There is no evidence that the populations had selective pressures from infant mortality rates and placental malarial infections.

A difference in ACPR could be attributed to a genetic difference between
the indigenous and non-indigenous populations. Humans have adapted several mutations to protect the carrier from malaria. There are three major mutations; The Duffy negative, sickle cell, and G6PD. The Red Blood Cell Duffy antigen negative (Duffy negative) is a random genetic mutation that occurs on the antigen receptor on the hemoglobin molecule. The mutation closes the portal, disallowing the parasite to enter. While there is no negative consequence to this mutation, it is only effective against P. vivax. This mutation is widespread in Africa, with 97% of the west and central Africa populations expressing it (Carter and Mendis 2002).

Sickle cell is another mutation that prevents falciparum infections. Heterozygous carriers for the sickle cell trait have an advantage in that carriers of the trait have one-tenth the risk of death. Unfortunately being homozygous for the trait causes sickle cell anemia and death without medical treatment. As a result this mutation has a high cost in the population in which it has been established (Carter and Mendis 2002).

The last possible mutation is the G6PD deficiency, an x-chromosome linked mutation. This mutation first became established in the west Mediterranean area. G6PD deficiency prevents the establishment of falciparum in the blood cells, at the cost of the host’s cell. Heterozygotes and homozygotes showed a 46-58% reduction in the risk of severe malaria (Ruwende, et al. 1995). South Asian countries like Bangladesh have very low frequencies of G6PD deficiencies (Rai and Kumar 2012).
Treatment failure of malaria is unlikely to be the cause of immunity through prior exposures or a genetic mutation. While it is possible for there to be a genetic mutation or a selection effect in the indigenous population, there is no evidence of this. One explanation for the difference in treatment failure is the microbiome. As the microbiome is influenced by diet and antibiotics, cultural-social differences between the two groups would lead to different microbiomes. A microbiome that is dysbiotic will be more likely to have deleterious effects on the immune system, leading to treatment failure.

Drug Seeking Behaviors

Non-indigenous Drug Seeking Behavior

The drug seeking behaviors of the non-indigenous populations and the inappropriate use of antibiotics may create a disruption of the microbiome leading to treatment failure. Non-indigenous populations of Bangladesh have more exposure to sub-therapeutic harmful doses of antibiotics, a problem stemming from problems with medical systems, lack of legislation, and poverty. The non-indigenous use of antibiotics has the potential to create prolonged problems of dysbiosis.

Bangladesh’s drug market lends itself to overuse of therapeutic drugs. Antibiotics are sold over the counter or without a prescription at the high rate of 86% (Morgan, et al. 2011). Bangladesh’s drug market is also flooded with counterfeit drugs. Counterfeit drugs do not have the proper dosing or
compounds due to a lack of regulations. As of 2006, there are over 70,000 illegal drug stores in the country of Bangladesh. Illegal stores sell fake, degraded, smuggled, and substandard medications (Islam 2006).

Even when drugs are prescribed by a doctor, often the wrong drugs are prescribed. Due to an overcrowding of the medical system, doctors often must diagnose a patient in less than 1 minute (the mean time was 54 seconds per patient) and frequently misdiagnose symptoms (Islam, 2008). Compounding this issue is the fact that there is no legislation limiting what doctors can prescribe for common or serious ailments. A survey of practitioners shows a 60% rate of antibiotic prescription regardless of the illness (Islam, 2008). In Bangladesh, a survey of 2000 people over the course of one month demonstrated that 90% of drugs taken came from pharmacies without a doctor prescription. There was no consultation with a physician pre or post treatment (Istúriz and Carbon 2000). Due to poverty, 48% of the antibiotics purchased were for a dose of less than one day (Ahmed, Tomson, et al. 2005).

The cultural pressures on drug seeking behaviors and faults in the medical system of the non-indigenous population lead to antibiotic misuse. Doctors who do prescribe antibiotics, may be doing so regardless of the disease of their patients. When doctors are not involved, the non-indigenous population receives their medication without prescription in sub therapeutic doses. Misuse of antibiotics in such a way could lead to dysbiosis.
Indigenous Drug Seeking Behavior

Indigenous tribes have their own medical practitioners on whom they rely on for medical treatment. These practitioners formulate botanical treatments based on experience with different botanicals. The drug seeking behaviors of this population varies greatly from that of the non-indigenous, possibly leading to a reduced risk of dysbiosis from antibiotic use.

Lack of medical facilities and cultural beliefs pressure indigenous people to consult with their tribe healers (Hanif, et al. 2009). Some remedies are additions to the diet and used as a prophylaxis instead of being used post exposure (Kadir, Sayeed and Mia 2012). Herbal medicines include Veronica amygdalina, which is a nutraceutical used to cure malaria (Toyang and Verpoorte 2013). Clerodendrum Viscosum, Eclipta prostrate, Urena Lobata are all herbs which have some antimalarial properties and are utilized by the Rakhain tribe who inhabit the Chittagong Hill Tracts near Rangamati (Hanif, et al. 2009). The indigenous population has a large array of botanicals that are incorporated into the diet.

The indigenous population relies on botanicals for treatment. Botanicals are added to the diet and do not have the same effects as antibiotics on the microbiome. Because of these drug-seeking behaviors, the potential for antibiotic misuse is higher for the non-indigenous populations.
Bangladesh’s Microbiome

The limited amount of studies on the microbiome in Bangladesh impedes the differentiation of microbiomes between indigenous and non-indigenous populations. However some microbiome studies do highlight dysbiosis among mostly non-indigenous city slums. The dysbiotic microbome has deleterious effects on immunity. An impaired immune system will inhibit natural recovery from malaria, possibly leading to treatment failure.

A comparison between the microbiome in children living in the United States and children living in the city slums of Dhaka in Bangladesh, show a monthly flux of the microbiome in Bengali children. Bengali children had higher individual rates of variations of the gut flora of the microbiome. The microbiome of children in the United States stayed constant month to month (Lin, et al. 2013). Children living in a first world country with minimal exposure to antibiotics and enteric diseases have a microbiome that remains constant.

A study that compares the microbiome makeup between the non-indigenous and indigenous populations is nonexistent. Non-indigenous Bengali children’s microbiome is subject to their environment and culture, potentially being impacted by the misuse of antibiotics. Populations exposed to antibiotics regularly will have higher rates of pathogenic bacteria, dysbiosis, creating a deleterious effect on immunity and health outcomes.
Conclusion

The microbiome, being closely related to health and immunity, has the potential to create treatment failure when dysbiotic. The indigenous populations with a symbiotic microbiome will have heightened immunity, aiding natural immunological responses to malaria. The non-indigenous population being prone to a dysbiotic microbiome will have a compromised immunological response to malaria.

In the case study, the reasoning for increased treatment failure in non-indigenous could be related to the cultural beliefs surrounding drug seeking behavior and antibiotic use. A population that misuses antibiotics will have a dysbiotic microbiome. The healthy microbiome aids immune function and immunological responses, while a microbiome in dysbiosis cannot offer the same. It stands to reason that the indigenous populations would have lower rates of treatment failure from a heightened immunity. The non-indigenous population, with a weaker immunity would then have relatively higher rates of treatment failure.

Research on the microbiome in Bangladesh and its relation to treatment failure is in its infancy. Further research is required to determine the accuracy and degree of difference of the microbiomes between the indigenous and non-indigenous populations. As more relevant research is performed a link between microbiome dysbiosis and inhibition of immunological response to infectious disease is plausible.
Application and Continued Studies

Understanding of the far-reaching effects of the microbiome on immunity and immunological response to disease is limited. If dysbiosis from antibiotic misuse occurs in such a degree as to cause treatment failure, it has the potential to impact the health outcomes of many countries. Further, treatment failure from antibiotic use might influence disease in a population.

Antibiotic misuse causing treatment failure through a disrupted microbiome and the resulting negative immunological effects is an untested theory. While it hasn’t been proven that antibiotic misuse causes treatment failure, it has been shown to promote the growth of pathogenic bacteria in the microbiome (Looft, et al. 2012). The shift in the microbiome to pathogenic bacteria and a total absence of bacteria has been correlated with decreased immune function of an individual (Abt et al., 2012). Suppression of immune function disrupts the individual’s response to illness, inhibiting recovery and prolonging illness.

Only through the continuation of research can an understanding of antibiotics effects on the immune system be obtained. Antibiotic misuse is culturally mediated and varies by country (Kardas et al., 2007). Cultural differences in drug seeking behavior and antibiotic compliance incite antibiotic misuse. Countries that offer antibiotics over the counter or antibiotics prescribed by nonprofessionals have greater opportunities for misuse (Cook, et al. 1995). The importance isn’t the countries or cultures that have a tendency to misuse...
antibiotics, but the health and immunological outcomes these populations may be subjected to resulting from the antibiotic misuse.

**Application**

If proven credible, the theory of antibiotic misuse on immunological response could allow for new techniques when assessing treatment failure. On a broader scale the cultural information regarding drug seeking behavior and antibiotic misuse can be used in disease spread and control simulations. More countries are experiencing antibiotic misuse both from faults in regulations, prescription and compliance. This trend of antibiotic misuse will have a negative impact on the microbiome and immune system.

Inappropriate antibiotic use is a growing problem both inside and outside the United States. Since the discovery of penicillin, antibiotics remain a popular choice of treatment for bacterial infections. In a study performed from 2003-2005, the average child in the United States received 10-20 courses of antibiotics before the age of 18 years old (Sharland 2007). Prescriptions of antibiotics in cases of bacterial infections are reasonable, but an inappropriately prescribed antibiotic only serves a negative influence on the microbiome. In the United States antibiotics can be prescribed inappropriately or at improper doses (Sharland 2007). The inappropriate prescription of antibiotics could have the same deleterious effects as antibiotic misuse.

As well as prescribing antibiotics in inappropriate situations, antibiotics can
be prescribed in sub-therapeutic doses. Currently the treatment of infections is
often based on clinical experience, as there are no consistent well-defined dosing
regimens for antibiotics (Liu, Müller and Derendorf 2002). In a French study,
medical students were not confident with choosing the correct antibiotic dosage
and interval. These students were unaware that 80% of the antibiotics prescribed
were in outpatient settings (Dyar 2013). Both of these studies show the potential
for inappropriate antibiotic use. While physician prescription of antibiotics can
create a problem, the drug seeking behavior and the compliance of the
population can also create antibiotic misuse.

The failure to take the medication as an omission of one or more doses of
antibiotics is the most common mistake. In cases of a milder infection an
individual may stop medications due to the symptomatic relief (Kardas 2002).
Patients expected to see signs of clinical improvement after 3 days in a pan-
European study (Branthwaite and Pechere 1995). This failure to exceed
expectation could lead to non-compliance. Adverse side effects could be another
possible reason for the premature halt in taking medication. In an Iranian study,
a majority of the participants stopped treatment due to feeling better or feeling
worse (Moradi, et al. 2013).

Other issues with dosing and medication type influence compliance.
Large difficult to swallow pills also lead to non-compliance (Wandstrat and
Kaplan 1997). As dosing frequency increases the average compliance
decreases. In a study conducted on patients who were prescribed antibiotics in a
range of 1-3 doses per day, the higher the frequency of dose the more likely a
patient missed a dose. Of the patients taking medication three times a day 76.5% missed at least one dose regardless of the presences of electronic time measuring (Cook, et al. 1995). Misuse of antibiotics not only causes resistance but also affects the microbiome.

Antibiotic misuse occurs globally and can have lasting health effects on a population. The theory put forth in this paper, that antibiotic misuse influences the immune system negatively, can allow for understanding of decreased immune response or an increase in treatment failures globally. Antibiotics have a deleterious impact on the microbiome, resulting in an impact on immunological response. Continued studies are needed to determine the degree of impact of antibiotics on the microbiome the subsequent immunological impact. Analysis of the health consequences of the microbiome, and antibiotics impact on it can be added as a cultural variable in determining disease patterns.
References


Black, Robert E., Lindsay H. Allen, Zulfiqar A. Bhutta, Laura E. Caulfield,


