Industrial Scale Production of R21/Matrix-M Malaria Vaccine for Sub-Saharan Africa
(Technical Paper)

Analyzing the Impact of the 1996 Pfizer Meningitis Clinical Trial on Vaccine Hesitancy in Nigeria
(STS Paper)

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

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Introduction

Malaria is one of the leading causes of death in the world (Tuteja, 2007), with the number of cases increasing from 227 to 241 million from 2019 to 2020 (*Fact Sheet about Malaria*, n.d.). Malaria transmission is mediated by mosquitos and consequently is prevalent in regions around the equator, with tropic regions being endemic for the disease. The bulk of the worldwide malaria cases are in sub-Saharan Africa, where 25% of child deaths are due to malaria. In addition to resulting in severe medical conditions and death, malaria incurs over $12 billion annual cost to Africa (Tuteja, 2007). As a result, the World Health Organization (WHO) aims to reduce the global malaria burden by 90% by 2030 with a vaccine that has at least a 75% protective efficacy (World Health Organization. Regional Office for the Eastern Mediterranean, 2022). Currently, however, there are no approved vaccine technologies that meet WHO's threshold level of effectiveness. Recently, a novel malaria vaccine, R21/Matrix-M, has surpassed the threshold in clinical trials, suggesting that the vaccine is a promising candidate for widespread use (Datoo et al., 2022). For the proposed technical capstone project, the team will be designing an industrial-scale process to manufacture the R21/Matrix-M vaccine for delivery to countries in sub-Saharan Africa.

In considering the distribution of the malaria vaccine, several barriers in sub-Saharan Africa prevent individuals from receiving vaccines, including cost, misinformation, safety concerns, and general distrust of western institutions and pharmaceutical companies. Some of the distrust and concerns stem from western colonialism and the general mistreatment of Africans in sub-Saharan Africa (Ghinai et al., 2013; Jegede, 2007). For example, in 1996, Pfizer had an unethical clinical trial to test the efficacy of the antibacterial Trovax against meningitis, which ultimately concluded with the death and deformity of Nigerian children. Since then, the clinical trial has played a role in shaping vaccine hesitancy in particularly Nigeria (Leonhardt, 2021). To

better understand the role of the clinical trial in Nigerian vaccine hesitancy, as well as to gain insight into how we can override distrust to reach higher vaccination levels, I will analyze the interaction between the clinical trial and Nigerians using Actor-Network Theory. Ultimately, the results of both the technical vaccine solution and the analysis of vaccine hesitancy in Nigeria will aid in effectively combatting malaria in sub-Saharan Africa.

Technical Project

In 2019, there were 228 million reported cases and 405 thousand deaths as a result of malaria, which remains one of the leading causes of morbidity and mortality in the developing world. In particular, Sub-Saharan African countries carry the majority of the malaria cases caused by *Plasmodium falciparum* (Figure 1), the parasite implicated in over 90% of world mortality due to malaria. The disease is spread via the *Anopheles* mosquito vector, which allows the parasite to enter the bloodstream and lyse host red blood cells during replication (Talapko et al., 2019). The infection results in high fevers, nausea, and muscle pain, among other symptoms. Severe malaria cases may result in severe anemia, comas, and respiratory distress. Although the drug artemisinin can completely eradicate the infection from the blood, due to drug resistance, *P. falciparum* can persist in the blood asymptomatically, causing recrudescence and serving as a source of further parasite spread (Cowman et al., 2016). As a result, there is a need for an effective malaria vaccine.

Currently, the RTS,S/AS01 vaccine, developed by GlaxoSmithKline, is the only approved vaccine for malaria that was recommended for widespread use in endemic regions (D'Souza & Nderitu, 2021). The vaccine is a virus-like particle (VLP) that presents circumsporozoite protein (CSP), a protein on *P. falciparum* that is critical for infecting cells, by linking it to an unrelated antigen. AS01, a saponin-based adjuvant, is added to enhance vaccine

efficacy (Nadeem et al., 2022). Unfortunately, clinical trial data demonstrate that at 48 months following the initial three-dose vaccination, the vaccine has only a 36% efficacy in children (5-17 months at receipt of vaccine) and a 26% efficacy in infants (6-12 weeks at receipt of vaccine). The efficacy further declines over time (Olotu et al., 2016).

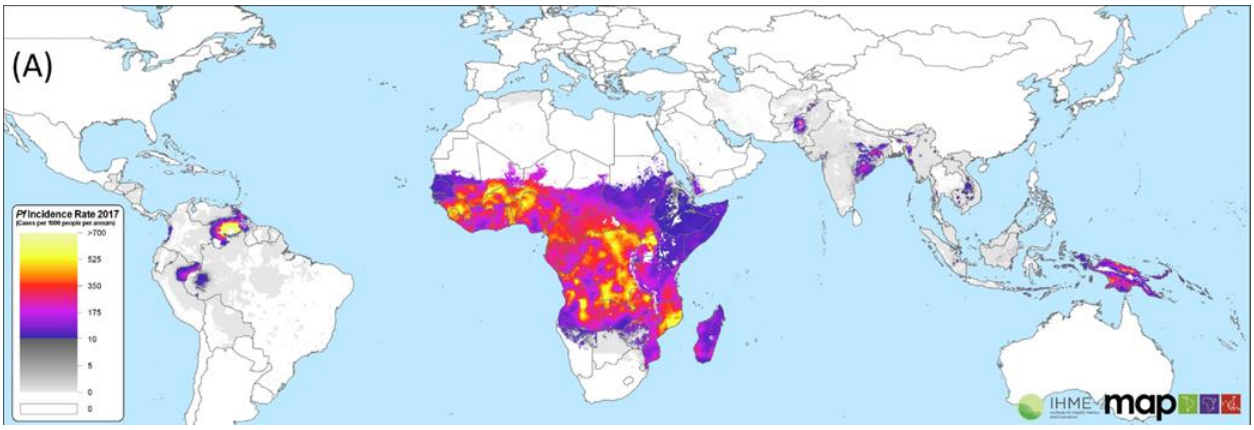

Figure 1. Frequency of *P. falciparum* Malaria Cases in 2017 (Price et al., 2020)

Note. This figure demonstrates that while there are cases elsewhere, the primary regions in the world with a high incidence rate of *P. falciparum* malaria are Sub-Saharan African countries.

Recently, scientists at Oxford University developed the first vaccine to meet the World Health Organization's goal for 75% efficacy. Known as R21/Matrix-M, the vaccine is a pre-erythrocytic malaria vaccine that improves the RTS,S/A01 vaccine design. By modifying vaccine synthesis to increase the proportion of CSP, Oxford scientists were able to develop a more immunogenic VLP (Collins et al., 2017). Recent clinical trial data shows that 24 months after initial vaccination, the R21 vaccine has an 80% efficacy against malaria in children (5-17 months at receipt of vaccine) when mixed with Matrix-M, another saponin-based adjuvant (Datoo et al., 2022). Additionally, R21/Matrix-M is easier to develop than RTS,S/A01, due to its cheaper and more modern design (Mandavilli & Cheng, 2022). Currently, the vaccine is manufactured by the Serum Institute of India, allowing for the production of R21 to be nearly 30

times greater than RTS,S (Ledford, 2022). Although the vaccine is still undergoing clinical trials to confirm efficacy, R21/Matrix-M is a promising candidate for widespread use.

In anticipation of vaccine approval, this technical project aims to develop a cost-effective process to manufacture single-dose R21/Matrix-M vaccines for use in preventing malaria infections in Sub-Saharan Africa. The process will involve industrial scale upstream, downstream, formulation and fill-finish stages (Figure 2). Upstream processing will include batch fermentation with *Pichia pastoris*, which is critical for R21 production. In downstream processing, the yeast cells will first be lysed using chemicals and bead vortexing, allowing the CSP fusion proteins to self-assemble into VLPs. Subsequently, the lysed material will go through centrifugation, followed by depth-filtration, and then two cycles of ultracentrifugation and size-exclusion chromatography to purify the particles (Collins et al., 2017). For formulation and fill-finish, the R21 protein particle will be mixed with Matrix-M, which will be acquired from Novavax, at a 1:10 ratio (Datoo et al., 2022), resulting in the final product. The project will conclude with an economic and feasibility analysis.

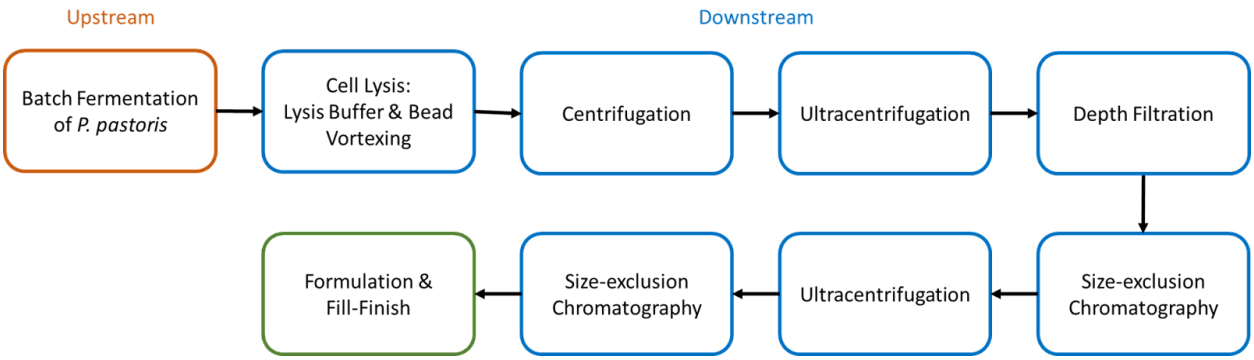

Figure 2. Simple Process Flow Diagram of Proposed R21/Matrix-M Manufacturing Process (Jayaraman, 2022)

Our team will complete the design project over two semesters in CHE 4474 and CHE 4476. We plan to meet weekly to review progress and assign future work. Additionally, we will

meet biweekly with Professor Eric Anderson, the chemical engineering capstone faculty advisor, to receive feedback and guidance. We will also consult Professor Michael King, an industry expert on vaccines, and Professor Giorgio Carta, a leader in bioseparations, for further advice. As R21/Matrix-M is a new vaccine still in clinical trials, there is limited available data on its large-scale manufacturing. As a result, our team will consult Collins et al., which documents the methodology for lab-scale vaccine production, and documentation on the production of VLP vaccines that use technologies similar to those in R21.

STS Project

In sub-Saharan Africa, child mortality is high, partially due to diseases that are preventable with vaccines. Of the sub-Saharan countries, Nigeria, with 14% of the world's unvaccinated children under five (Mahachi et al., 2022), consistently ranks as one of the top five countries with the highest child mortality for preventable diseases, including tuberculosis and meningitis (Vanderslott et al., 2013). This high mortality rate corresponds to high rates of vaccine hesitancy in Nigeria, where vaccine acceptance only ranges from 20% to 53% across the country (Olu-Abiodun et al., 2022). As a result, researchers have conducted various studies and analyses to determine the major causes of vaccine non-acceptance and hesitancy. These factors include the mistrust of the government and western institutions and the fear of vaccine side effects (Ghinai et al., 2013; Jegede, 2007). Each of these causes for hesitancy is influenced by the interaction between the sociopolitical landscape of Nigeria and Pfizer's meningitis clinical trial.

In 1996, Pfizer wanted to test the efficacy of its antibacterial medicine, Trovan, against a natural strain of meningitis in young children. Since, at the time, Nigeria was suffering from a meningitis epidemic, Pfizer looked to Kano, a northern Nigerian state, to hold its clinical trial of

the medication. However, the trial proved to be disastrous. The clinical trial protocols that Pfizer employed did not adhere to the proper standards that would ensure patient safety. Additionally, there was no informed consent that Nigerian patients were taking an experimental drug and that there was another drug, proven to be effective against meningitis, offered locally (Stephens, 2000). Ultimately, 11 children died and more developed life-changing disabilities upon the conclusion of the trial. Consequently, Nigerian families sued Pfizer, which eventually settled out of court without ever admitting liability. The actions of Pfizer likely contributed to the perception that western medical institutions and products are unsafe (Pertwee et al., 2022). For example, a farmer from Kano stated that Nigerians "cannot trust the white man or our federal government because many years ago they were in partnership when they brought medicine to poison our people" (Yahya, 2006). These views of mistrust helped motivate the low initial vaccination rates for polio (Ghinai et al., 2013) and COVID-19 (Pertwee et al., 2022) in Nigeria. Understanding how exactly the clinical trial, in interaction with other variables present at the time, produced vaccine mistrust will provide valuable insight into how to design effective vaccination campaigns in both Nigeria and sub-Saharan Africa in general.

To analyze the role of the Pfizer clinical trial in developing vaccine hesitancy in Nigeria, I will use Actor-Network Theory (ANT). ANT is a sociotechnical framework that enables the user to study the relationships between different actors, which may be animate or inanimate (Cressman, 2009). Specifically, this allows us to analyze how the connections between the actors help shape social features of society (Cresswell et al., 2010), or vaccine hesitancy in Nigeria in the case of the present proposed research. Moreover, this method of analysis will lay the framework to assess how we can destabilize the network (London & Pablo, 2017) to minimize vaccine hesitancy. Common criticisms of ANT include the fact that there can be an infinite

number of actors and, as a result, multiple ways to interpret a network (Cressman, 2009). As a result, I will refine the ANT analysis to examine a few key actors, including, but not limited to, Nigerians, the Nigerian Government, vaccines, and Pfizer.

To determine how the Pfizer meningitis clinical trial has impacted Nigerian vaccine hesitancy via ANT, I will perform network analyses on the relevant connections between the actors in the system. To illuminate basic connections relating to the clinical trial, I will refer to *The Washington Post*'s multi-article reporting of the controversy (Stephens, 2000). For more nuanced information, I will gather scholarly articles and studies regarding social, political, and cultural factors that influence vaccine hesitancy towards polio and COVID-19 vaccines in the 21st century. Specifically, I will conduct literature searches using combinations of keywords like 'vaccine,' 'mistrust,' 'polio,' 'covid,' 'Nigeria,' 'Pfizer,' 'meningitis,' 'culture,' and 'politics.' I will organize these sources based on the relevant actor and use the information to infer connections. The final deliverable will entail an ANT analysis of the vaccine mistrust network in Nigeria and provide a brief analysis of the efficacy of current vaccination campaign techniques in Nigeria based on their ability to destabilize the proposed network.

Conclusion

For the technical project, we aim to develop an industrial-scale process to manufacture the highly effective R21/Matrix-M malaria vaccine for distribution in sub-Saharan Africa. The process will include upstream processing to produce the necessary fusion protein, downstream purification to recover and achieve high levels of purity of the VLP, and formulation and fill-finish to add the Matrix-M adjuvant and process the vaccine for distribution. The final deliverable will provide the necessary information to build the processing plant and produce the vaccine, which will help provide immunity against malaria and decrease child mortality rates in

sub-Saharan Africa. The STS project will employ ANT to illustrate how the 1996 Pfizer meningitis clinical trial influenced vaccine hesitancy in Nigeria. The generated network will provide a framework to analyze how vaccination campaign techniques across sub-Saharan Africa destabilize the vaccine hesitancy network and will ultimately inform the best practices for a malaria vaccination campaign with our technical vaccine product.

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