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INTRA PRISON TRANSMISSION AND GENETIC DIVERSITY OF HEPATITIS B VIRUS IN A GHANAIAN PRISON

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL MEDICAL MICROBIOLOGY DEGREE.

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DECLARATION

I, Nancy Nartey hereby declare that this study was done by me towards the award of Master of Philosophy in Medical Microbiology under the supervision of Rev. Fr. Prof. Kwamena W.C Sagoe and Professor Julius A. A. Mingle all of the Medical Microbiology Department, School of Biomedical and Allied Health Sciences University of Ghana. All document used in the course of this research have been duly accredited.

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DEDICATION

This work is dedicated to my late father Robertson Doku Nartey, my foster father Emmanuel Nartey, my mother Charlotte Quansah, my brother, Ishmael Nartey and to my entire family and all the wonderful people who helped to make this work a success.

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LIST OF ABBREVIATIONS

aa	amino acid
ATG	Alamine Thymine and Glycin
cccDNA	Covalently closed circular DNA
CDC	Center for Disease Control and Prevention
EMI	Emergency Management of Injuries and Post-exposure Prophylaxis
ER	Endoplasmic Reticulum
HBcAg	Hepatitis B virus Core Antigen
HBe	Hepatitis B virus e protein
HBeAg	Hepatitis B virus E antigen
HBsAg	Hepatitis B virus Surface Antigen
HBV	Hepatitis B virus
HBxAg	Hepatitis b X antigen
HSPGs	heparin sulfate proteoglycans
MEGA	Molecular Evolutionary Genetics Analysis
MHR	Major Hydrophilic region
NTCP	sodium-taurocholate cotransporting polypeptide
NTD	N-terminal assembly domain (NTD)
OP	Outside Prison
ORF	Open Reading Frame
PCR	Polymerase chain reaction
Pol	polymerase
RT	Reverse transcriptase
S	Surface gene
SADC	Southern African Development Community
SHB	Small Hepatitis b surface antigen protein
TI	Tattoo Implement
WP	Within Prison

ABSTRACT

Background: Selecting an ideal genetic regions for the phylogenetic analysis of hepatitis B virus (HBV) transmission continue to be a matter of debate, with different researches preferring different genomic region. Whole genome sequence analysis is always the gold standard for this this kind of research. But in middle income countries such as Ghana, where HBV infections are endemic, it is almost impossible to study a large number of samples because of financial constraints. Thus, analysis of nonoverlapping, fast-evolving regions was recommended.

Aim: This study determined the intra prison transmission and genetic diversity of hepatitis B virus among inmates in correctional facility in Ghana

Methodology: Hepatitis B surface antigen (HBsAg) positive archived plasma samples from a Ghanaian prison which was part of a nationwide survey of HIV and HBV in correctional facilities was used for this study. HBV DNA was extracted from 26 archived plasma samples and the precore, core region, surface envelope gene and the preS1 and preS2 regions amplified. The Sanger method was used to sequence the S gene of 17 plasma samples, Precore/core gene of 8 samples and PreS1, PreS2 gene for 5 samples. Sequence Identity Matrix, phylogenetic analysis and mutational analysis were used to establish possible intra prison transmission within the selected prisons.

Results: Sixteen out of the 17 successfully sequenced samples belonged to HBV genotype E with the exception of one sample (14S) harboured the A/E recombinant. Sequences from the same prison clustered together and identity matrix revealed a narrower similarity range for these

sequences. Surprisingly sequences from the two different prisons that were added to the sample size of 24 were closely related to each other and also to some of the sequences of the major prison. Mutations were seen across all the regions of the genome that were amplified however this were not uniformly distributed in the samples. Sequences with Sequence Identity Matrix value close to 1 and were strongly related to each other using phylogenetic tree had common mutations across the various regions investigated. Of note is the presence of L209V mutation, L217R mutation and the vaccine escape mutation (M133L) in the S gene and W28* in the precore gene of two inmates.

Conclusion: Recent infection, coupled with a similarity index value close to 1, clustering of sequences together and demographic data suggest that there is on-going intra prison transmission of HBV within inmates. Presence of common mutations may also be used as a means of establishing horizontal transmission within prison.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Hepatitis B virus (HBV) infection is a weighty and widespread communicable disease of the liver, distressing millions of people worldwide. During the acute infection stage, most individuals do not experience any sign nonetheless some individuals develop acute illness. Symptoms exhibited during the acute stages include extreme fatigue, jaundice, nausea, dark urine, abdominal pain and vomiting (WHO, 2017). A small percentage of individuals with acute hepatitis may develop acute liver failure, which can tether to death. The chronic stage (hepatitis b surface antigen (HBsAg) persistence for at least 6 months) of the disease may be associated with cirrhosis and hepatocellular carcinoma (WHO, 2017). HBV remains potent outside the body for at least 7 days during which it can still cause infection if it finds its way into the body of a person who is not protected by the vaccine. The average incubation period of the virus is 75 but this can also fluctuate from 30 – 80 days. After HBV infection, the virus can be detected within 30 - 60 days (WHO, 2017). The aftermath of the disease after infection is governed by the host immune response. Menace for chronic infection is inversely related to age at primary infection with approximately 90% of infected infants and 30% of infected children aged <5 years becoming chronically infected, compared with 2%–6% of adults. In the case of African countries, 20%-30% of individuals infected by HBV during childhood become chronic carriers and only 10% of such people linger HBeAg positive during adolescence. A large number of HBeAg positive individual rapidly lose HBeAg, at a yearly rate of 14%-16% (Zampino *et al.*, 2015a). Among persons with chronic HBV infection, the peril for premature death from cirrhosis or hepatocellular carcinoma is 15%–25% (Centers for Disease Control and Prevention, 2012).

Approximately 257 million people globally are living with hepatitis B virus infection and over 686 000 individuals die each year as a result of the complications like cirrhosis and hepatocellular carcinoma presented by hepatitis B (WHO, 2017). The global prevalence of chronic HBV infection varies widely, from high (>/=8%, e.g., Africa (precisely sub Saharan countries like Burkina Faso, Gabon, Nigeria Cameroon and Namibia), Asia and the Western Pacific). Areas of intermediate endemiciy (2-8% include; Southern, Eastern Europe and other African counties like Senegal, Zambia, Liberia and Serra Leone). Whereas areas of low endemicity (<2%, include; Western Europe, North America, Australia and African countries such as Algeria, Egypt, Tunisia and Morocco) (WHO, 2015; Zampino *et al*., 2015; Hou, Liu, & Gu, 2005). The reported prevalence of HBV infection in the general population ranges from 9-12% (Adjei *et al*. 2008). According to Zahn *et al*. (2008), in Ghana HBV chronically infects 15 % of the populace. Before age 40, almost everyone has come into contact with the virus and tested positive for antibodies against the HBV core antigen (anti-HBc). Currently the disease burden has reduced with the inception of the widespread hepatitis b virus vaccination programs which started in the early nineties (Zampino *et al*., 2015a).

HBV exist in nearly all body excretions nonetheless secretions that pose a risk include blood, body fluids having noticeable blood, vaginal secretions and semen (EMI, 2016) . The virus is found in highest concentrations in blood and in some cases wound exudate and in other body fluids such as semen and vaginal secretions the viral concentrations are lower (EMI, 2016; Hepatitis B Foundation, 2015). HBV is successfully transmitted by percutaneous or mucous membrane exposure to infectious blood or body fluids that contain blood (Hepatitis B Foundation, 2015). The major means of transmission involve close household or sexual contact

with an infected individual, intravenous drug use, perinatal mother to child transmission and nosocomial exposure (EMI, 2016). Percutaneous exposures that have resulted in effective transmission of the virus include sharing unsterilized injection needles for intravenous drug use, transfusion of unscreened blood or blood products, tattooing, haemodialysis, acupuncture and wounds from sharp instruments fouled with infected blood (WHO, 2017; EMI, 2016). The mode of transmission differs depending on the endemicity of the HBV infection. In areas of high endemicity, mother to child transmission is the main mode of transmission, whereas in areas of low endemicity, sexual contact amongst high risk individuals is the predominant route (Utsumi & Lusida, 2015; Hou, Liu & Gu 2005). The most important risk factors that have been associated with HBV infection include; unprotected sex with an infected partner, mother to child transmission, men who have sex with other men and sharing of intravenous drug needles (WHO, 2017; EMI, 2016; Utsumi & Lusida, 2015; CDC, 2015). Horizontal transmission within correctional facilities and from mother to child have also been established using phylogenetic analysis (SADC, 2009; Adjei *et al*., 2006; Khan *et al*., 2005; Oon, Chen and Koh, 2000).

Infection with HBV can be diagnosed by testing blood of patients for the presence of viral markers such as the HBsAg, and HBcAg. HBeAg suggest high level of infectivity and high level of viral replication and seroconversion from HBeAg to anti-HBe usually indicates a low level of viral production and low serum HBV DNA levels. Hepatitis B disease control measures include immunization which appears to be the most effective and cost-saving means of prevention. However some countries are unable to implement vaccination programs because the health care institutions and the government is encumbered with treatment and management of the infection as well as its preclusion(Zampino *et al*., 2015a). This has resulted in the continuous wide spread of the virus in developing countries. The vaccine since 1982 has had an excellent record of

effectiveness and safety and in most cases one of the following two options is recommended by WHO and in most cases, 1 of the following 2 options is considered appropriate:

- A 3-dose schedule of hepatitis B vaccine, with the first dose (monovalent) being given at birth usually within 24 hours and the second and third (monovalent or combined vaccine) given at the same time as the first and third doses of pertussis (whooping cough), diphtheria and tetanus – (DTP) vaccine; or
- A 4-dose schedule, where a monovalent birth dose is followed by three monovalent or combined vaccine doses, usually given with other routine infant vaccines. Protection lasts for at least 20 years and is probably lifelong (WHO, 2017).

It is also imperative to educate high risk groups as well as heath care workers about the modes of transmission and the dangers associated with the disease as this will reduce the chances of transmission. There is also the need to screen donated blood and blood products to reduce transmission (WHO, 2015). Presently, treatment for individuals with chronic hepatitis B consists of two types of drugs namely immunomodulators, such as interferon alfa, peginterferon and nucleotide analogues (NAs) such us adefovir, entecavir, lamivudine, telbivudine and tenofovirn (Chang & Suh, 2008). Tenofovir or entecavir are the potent drugs mostly recommended by World Health Organizaton (WHO) to subdue hepatitis b virus. This is because they seldom lead to drug resistance as compared with other drugs, have little side effects so necessitate only limited monitoring and these are easy to take (1 pill a day). It is worth noting that treatment does not cure hepatitis b infection rather it only suppresses viral replication and thereby slow the progression of cirrhosis, reduce incidence of liver cancer and overall increase long term survival of infected individuals (WHO, 2017). The lack of editing function by HBV reverse transcriptase (HBVrt) leads to constant substitution of nucleotide or nucleoside with substitution rates of 4.2.

The end result is the emergence of several genotypes, subgenotypes, mutants and recombinants (Sunbul, 2014). HBV mutants can be found in HBsAg, Basal core promoter/precore gene and X gene (Lazarevic, 2014).

HBV mutants also arise as a result of factors such as host immunity (endogenous pressure), and vaccine or antiviral agents (exogenous pressure). It has been established that mutations that occur in the genome of HBV such as surface (S), precore (PC) and basal core promoter (BCP) genes do not only influence the replication fitness of the virus but can also impact the disease outcome and its response to treatment (Kidd-Ljunggren *et al*, 2002)

1.2 PROBLEM STATEMENT

As pertains to other countries across the globe, the incidence of HBV infection among prison inmates has always been higher when compared with the entire populace that reside outside the prison walls. The prevalence of HBV within Ghanaian prison has been reported to range between 13 – 17 % which is by far higher than the prevalence recorded in different populations including blood donors, pregnant women and the like in the general population (9 – 12%) (Adjei *et al*., 2008, Amidu *et al*., 2012, Walana. *et al* 2014). Even though insight pertaining to transmission dynamics of HBV infection is necessary in ascertaining the particular risk factors and putting in place measures to curb further transmission, few studies in this high risk population has been conducted in Ghana. Ongoing transmissions have been speculated by many researchers home and abroad and this phenomenon is attributed to the practice of homosexuality, intravenous drug use among others (UNAIDS, 2016; Shewan, 2013; Adjei *et al*., 2006; *Taylor et al*., 2000; Hutchinson *et al*., 1998; Stark *et al*., 1997; Mutter *et al*., 1994).This speculations still need to be

validated as sparse knowledge on intraprison transmission is available in Ghana. Little work has also been done on characterization and mutational profiles of HBV strains and therefore there is limited data on mutations in the HBV surface (S), precore (PC) and basal core promoter (BCP), X and polymerase genes. These mutations are known to adversely affect the clinical outcome of HBV disease. It is however unclear if specific mutations can be used to establish horizontal transmission. A study on transmission of HBV using the S gene in 26 HBsAg positive archival samples of a particular prison in Ghana has suggested possible transmission and revealed some recombinant strains (unpublished data).

1.3 JUSTIFICATION

The prison population in Ghana comprise of inmates from different religions, ethnic groups, regions and neighboring countries like Nigeria, Togo, Niger, Guinea, Mali Liberia among others including China, India and Thailand (GPS 2013). This inmates although appear hidden, are high risk populations that need to stay healthful before coming back into the community in order to reduce further spread of HBV infections. It is therefore in it right place for this study to be conducted in order to validate transmission dynamics within the prison setting and instigate the provision of prevention and treatment supports to inmates not only because it's their health right but because it will be of benefit to the community at large (Kamarulzaman *et al*., 2016). It is vital to comprehend the intra prison transmission dynamics and genomic diversity of HBV since these have clinical implications. This research is a follow-up of a study which revealed typical mutations across the s-gene of HBV strains found in prison inmates that formed the population to be used in this study. This research will provide data on variant HBV strains possibly circulating among prisoners and may provide information on the horizontal transmission of HBV.

1.4 AIM

The overall aim of this research is to determine the intra transmission and genetic diversity of HBV in a prison.

1.5 SPECIFIC OBJECTIVES

- To establish relatedness of HBV strains in prisoners using the S gene, Precore/Core gene and the PreS1/PreS2 gene.
- To determine if specific mutations may be used to establish horizontal transmission.

CHAPTER TWO

LITERATURE REVIEW

2.1 HBV STRUCTURE, GENOME ORGANISATION AND PROTEINS

The hepatitis B virus (HBV) is 42nm enveloped DNA virus and belong to the family hepadnaviridae. Compared to all the DNA viruses, it is known for its small genome size of 3.2 kb (Shuping Tong, Li, Wands, & Wen, 2013). It has a partially double-stranded HBV genome sheathed within a core particle that is covered by host derived lipid containing envelope. According to Suppiah *et al*. (2014) the partially double stranded 3.2 kb DNA has four open-reading frames (ORF) namely the C, P, S and X. The C (core) gene is divided into the precore region (29 amino acid codons) and the core region (181 codons) by 2 in-frame initiating ATG codons and codes for the hepatitis b core protein (HBcAg) and hepatitis b e protein (HBeAg) depending on whether translation is initiated from the core or precore regions, respectively (Fig. 1B). HBcAg contains 183 or 185 amino acids (aa) for most of the genotypes, but genotype G is lengthier (195 aa) because it has an extra interior sequence (HBVdb, 2017). The core protein has an intrinsic capacity to self-assemble to form a capsid-like structure which contains a well basic cluster of amino acids at its C-terminus and an RNA-binding activity (Liang 2010). The N-terminal assembly domain (NTD) is involved in core particles assembly (Figure 2) and the C-terminal functional domain which is arginine-rich is involved in packaging of the pregenome/reverse transcriptase complex (HBVdb, 2017).

The precore open reading frame (ORF) codes for a signal peptide that guides the translation product to the endoplasmic reticulum, and it is at this point that the protein is additionally handled to form the HBeAg which is exuded into the blood. HBe protein is translated from the first ATG of core ORF, and so it has an N terminal extension of 29 amino acids. This is a

hydrophobic province which creates a signal peptide that guides the protein to the endoplasmic reticulum. The protein is transported via the Golgi to the cellular surface during which the basal tail and the signal peptide are removed. The HBeAg (matured protein), is secreted as a monomeric protein, which performs a function in the immune system escape (HBVdb, 2017). The P gene, encrypt DNA polymerase (pol) which is functionally separated into three domains; the terminal protein domain, the reverse transcriptase (RT) domain and H domain (Liang, 2010). The surface envelope (S) gene codes for the viral surface envelope proteins (HBsAg) and the X gene ciphers the regulatory protein (HBxAg). The S ORF is organizationally alienated into the pre-S1, pre-S2, and S regions (figure 1). All the envelope proteins of HBV contain highly immunologic HBsAg that encourages humoral immunity. HBsAg, varies antigenically with a shared antigen designated a, and two pairs of mutually exclusive antigens, d and y, and w (which includes numerous sub determinants) and r, ensuing 4 main subtypes (adw, ayw, adr and ayr). Since there is shared determinants, fortification against one subtype bestows protection to the other subtypes (Mohammed & Eldaif, 2014). The HBsAg ‘‘a’’ determinant (amino acids 124–147) is located within the major hydrophilic region (MHR amino acids 99–169) of the surface protein (Velu *et al*., 2008). The dispersal of the subtypes varies across the globe (Mohammed & Eldaif, 2014).

The P gene overlaps with the 3’ end of the core gene, the entire envelope gene, and the 5’ end of the X gene (figure 1). HBV employs the use of 4 promoters together with imprecise transcription initiation sites to generate six of its seven proteins. These include the X promoter for the 0.7 kb RNA encoding Hepatitis B virus X (HBx), the L promoter for the 2.4 kb RNA encoding the L protein, the S promoter for the 2.1 kb RNA giving rise to the M and S proteins, and the core

promoter for the 3.5 kb RNA for the precore/core and core proteins (figure 1.1) (Tong, Li, Wands, & Wen, 2013).

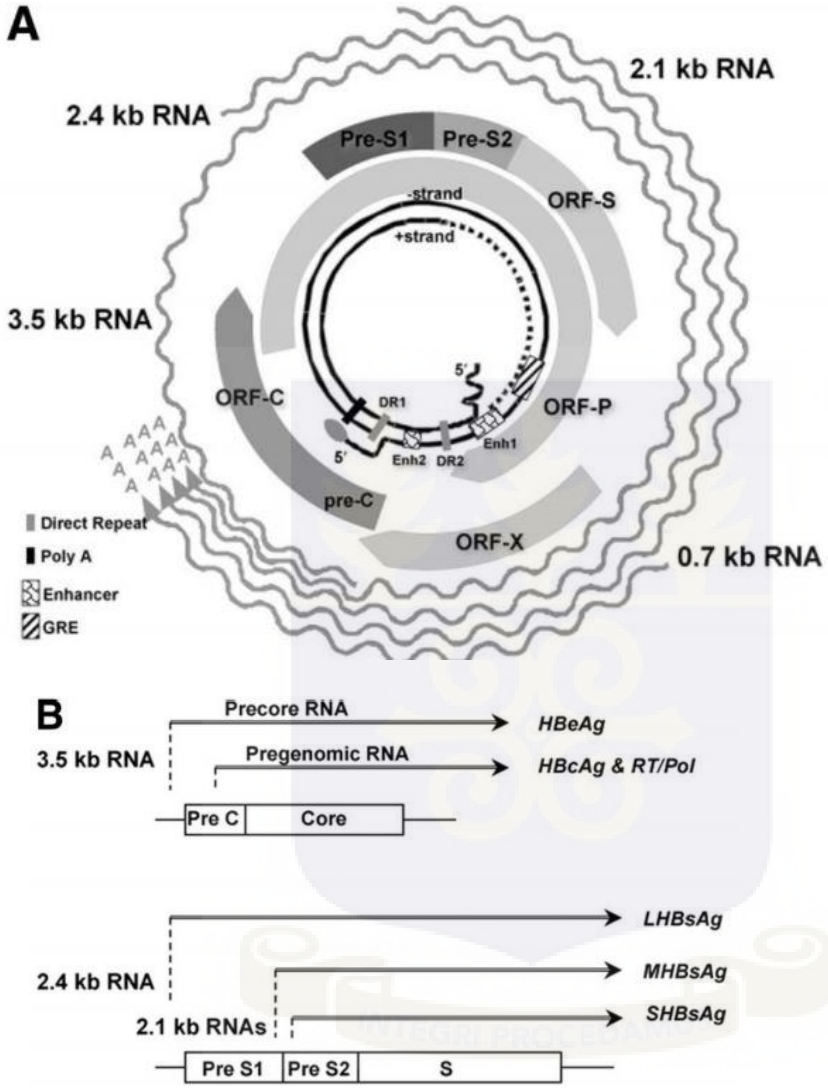

Figure 2.1: The HBV genome. (A) The genomic organization, RNA transcripts and gene products are shown with several key regulatory elements. (B) The transcription start sites of various HBV transcripts and the proteins they encode

(Source: Liang, 2010).

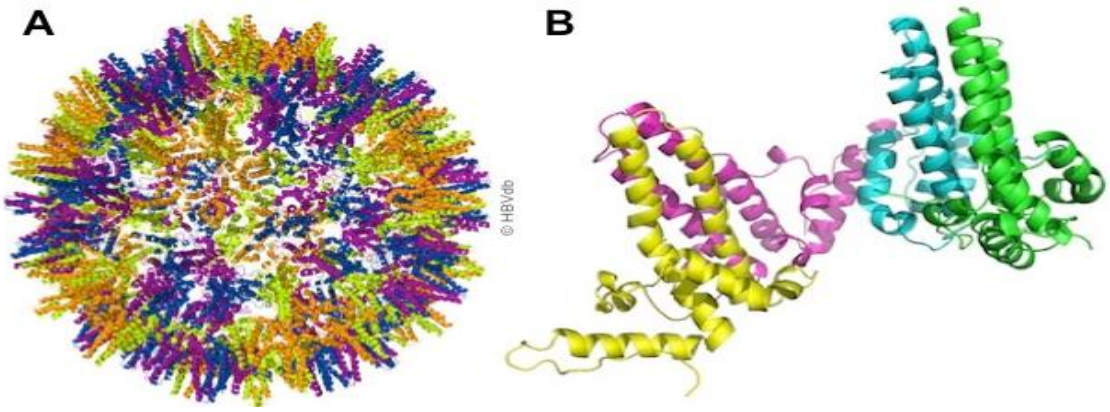

Figure 2.2: Core protein structure. (A) The core assembly in capsid (model of 120 dimers). (B) The structure of core subunit (T=4, chain A,B,C,D). (HBVdb, 2017).

2.2 LIFE CYCLE OF HBV

The preS1 region of the large envelope protein of HBV has been acknowledged as the key factor that facilitates the attachment of the virus to the cell surface heparin sulfate proteoglycans (HSPGs) and also aids in the binding of the virus to the specific receptors on hepatocytes (Schulze, Gripon & Urban 2007). Current studies have demonstrated that sodium-taurocholate cotransporting polypeptide (NTCP) is a functional receptor in HBV infection (HBVdb, 2017). The role played by preS1 domain in the initial steps of viral contact with hepatocytes and cell entry was confirmed using synthetic peptides obtained from the preS domain that repressed virus infection (Urban Bartenschlager & Kubitz 2014). Once the virus enters the liver cell, it uncoats and makes its core particle accessible. This makes it possible for the viral DNA to be delivered to the nucleus of the hepatocytes where viral plus strand DNA synthesis is accomplished by ligation of the open DNA strand. This results in the formation of covalently closed circular DNA (cccDNA) which serves as a template for RNA viral transcription. This prototype for viral production acts as a reservoir for successive recurrence of formerly cleared or treated infection ((HBVdb, 2017); Asmuth *et al*. 2004). The 4 viral RNA transcripts created from this template are conveyed into the cytoplasm, where translation of viral proteins and assembly of the viral

capsid occur. Capsid assembly embroils reverse transcription of the viral RNA to the viral DNA and it is the goal of therapeutic intervention. Consequent movement of the capsid and the residual viral proteins into the Golgi apparatus and the endoplasmic reticulum results in final virion assembly and secretion of mature viral particles. This secretory pathway describes the noncytopathic nature of HBV infection (Asmuth *et al*., 2004). On the other hand, after reverse transcription and completion of viral DNA replication, HBV again crosses the nuclear membrane to amplify the covalently closed circular DNA (cccDNA) pool which continues with a variable half-life, and possibly be transferred to daughter hepatocytes after mitosis. Thus, the hepatocyte life span and viral kinetics will influence considerations regarding duration of therapy (Asmuth *et al*., 2004). Figure 2.3 illustrates the life cycle of HBV.

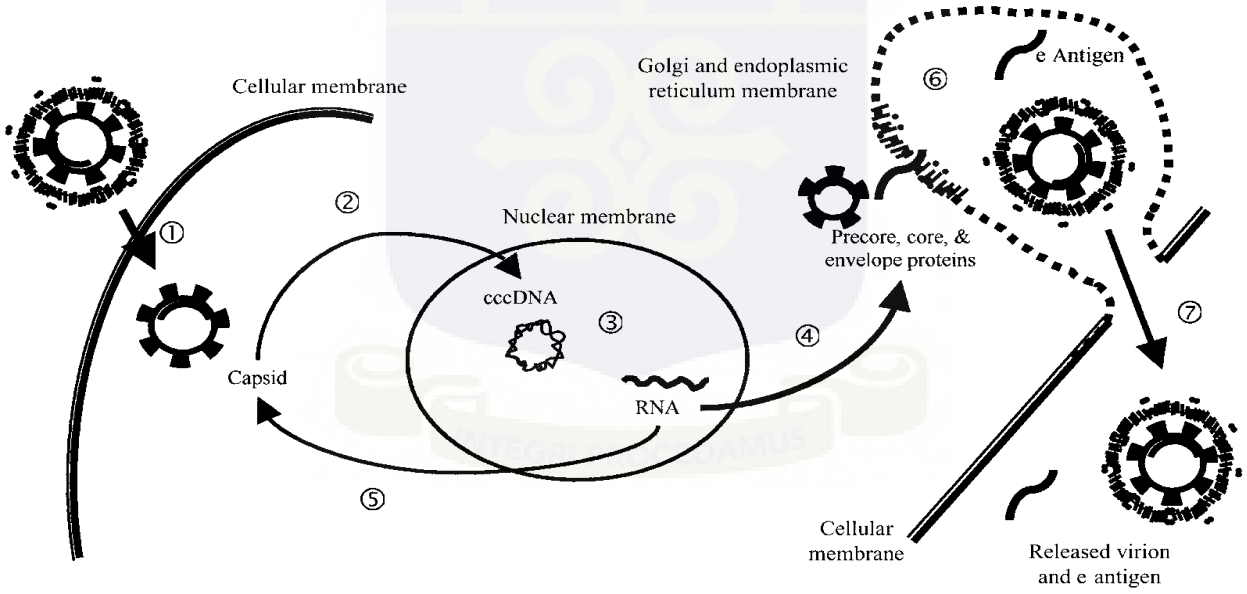

Figure 2.3: Replication cycle of the hepatitis B virus: step 1, viral entry; step 2, uncoating, completion of DNA plus-strand, and transnuclear membrane migration where covalently closed circular DNA (cccDNA) is formed; step 3, mRNA transcription from cccDNA; step 4, viral protein translation and capsid assembly in the cytoplasm; step 5, viral capsid and pregenomic

RNA replenish the pool of cccDNA; step 6, assembly of viral particles in the Golgi apparatus and the endoplasmic reticulum; and step 7, noncytopathic release of virions.

2.3 HEPATITIS B SEROLOGICAL MARKERS

To diagnose HBV infection and its related diseases, more than one test is available and this include a collection of biochemical, clinical, serological and histological findings (Liang, 2010). Most of which is based on serology where an individual's blood is tested for the presence of antibodies or antigen. It is important to clearly understand and interpret serological results for proper diagnosis of the various clinical forms of HBV infection (Table 2.1). Antibodies are formed by the host immune system in the presence of an antibody to neutralize its effect and destroy the pathogen (Cutler, 2013; Liang 2010). Antibodies are produced to fight infections therefore the presence of antibodies in the blood simply means that one has been exposed to the virus in one way or the other. Same can be said about an antigen being present in one's blood which is indicative that the virus is present in the individual. Different serologic markers are used to determine the various phases of the infection. There are six markers to look out for to be able to determine whether an individual has been recently infected, is a chronic carrier or has a past infection as a result of natural exposure to the virus or by vaccination (EMI, 2016; Cutler, 2013). These markers include the following.

Hepatitis B surface antigen (HBsAg): This is the protein at the exterior part of the virus and during an acute or chronic phase of infection higher concentrations can be detected in the serum of a patient. It is usually the first serologic marker to appear during a recent acute infection. After being exposed to HBV infection HBsAg can be detected as early as one to two weeks or as late as eleven to twelve weeks and its persistence after six months is a marker of chronic infection

(Liang, 2010). When an individual test positive for HBsAg it is a signal that the person is has been exposed to the virus. As part of the body's immune response to fight against infections, antibodies to HBsAg are produced by the body to help combat HBV infection. HBsAg is the antigen of interest when making hepatitis b vaccine.

Hepatitis B surface antibody (anti-HBs): The presence of anti-HBs is indicative of immunity and recovery hepatitis B virus infection. Individuals that have been effectively vaccinated against hepatitis B also develop anti-HBs. This antibodies appear late during infection, typically during recovery or convalescence after clearance of HBsAg. Nevertheless, between 10% and 15% of individuals who recover from hepatitis B do not develop detectable anti-HBs rather they have anti-HBc alone as a marker of previous infection. It in for this reason that anti-HBc testing still remains the most reliable means of assessing previous infection with HBV, whereas anti-HBs testing is used as a measure of immunity and response to HBV vaccine (Liang, 2010).

Total hepatitis B core antibody (anti-HBc): This comes to play at the period when symptoms begin to manifest and remain for the rest of one's life. The first antibody to appear is immunoglobulin M (IgM) class, which reduces in titer as levels of IgG anti-HBc arise. To test positive for anti-HBc suggest forgoing or ongoing infection with hepatitis B virus in an indeterminate time frame.

IgM antibody to hepatitis B core antigen (IgM anti-HBc): Its presence is indicative of an acute infection. Positivity indicates recent infection with hepatitis B virus ($\leq$ 6 months).

Hepatitis B envelope antigen (HBeAg or HBe): It is a measure of high viral replication and infectivity. It can present in the blood during both acute and chronic hepatitis b infection and an individual who test positive for this marker is highly infectious.

Hepatitis B envelope antibody (HBeAb or Anti-HBe): It is produced shortly after clearance of HBeAg, usually at the peak of HBV infection. When on seroconverts from HBeAg to HBeAb that individual most likely to attains long term clearance of the virus and initiation of recovery. Within a few weeks after viral markers appear, rise in aspartate aminotransferase and serum alanine levels occurs and this is the period where jaundice possibly manifest. HBV DNA testing can also be useful in the assessment of level of viral replication and has over the time been useful in diagnosis and management of HBV infections. Real-time polymerase chain reaction–based assay (TaqMan) for instance has a lower limit of detection of 5–10 HBV DNA copies/mL and with this scale of sensitivity it can precisely quantify wide ranging levels of HBV DNA even before other serological markers manifest (Liang, 2010). The presence of high levels of HBV DNA even after resolution of hepatitis may suggest failure to manage the infection development of a chronic infection.

Table 2.1: Summary of hepatitis b serological markers and their interpretation

Hepatitis B serological Markers	**Test Result**	**Interpretation**
HBsAg anti-HBc anti-HBs	Negative Negative Negative	Susceptible
HBsAg anti-HBc anti-HBs	Negative Positive Positive	Immune due to natural Infection
HBsAg anti-HBc anti-HBs	Negative Negative Positive	Immune due to hepatitis b vaccination
HBsAg anti-HBc IgM anti-HBc anti-HBs	Positive Positive Positive Negative	Acute Infection
HBsAg anti-HBc IgM anti-HBc anti-HBs	Positive Positive Negative Negative	Chronic Infection
HBsAg anti-HBc anti-HBs	Negative Positive Negative	Interpretation unclear. Four possibilities: - Resolved infection (most common) - False positive anti-HBc, thus susceptible - "Low Level" chronic infection - Resolving acute infection.

2.4 THE PRISON SERVICE AND THE PRISON ENVIRONMENT

In general prisons are divided into several categories relating to security classification of the prisoners it holds, age and gender. Security categories are also based on the type of crime committed, the length of sentence, the likelihood of sentence and the danger to the public if they were to escape. Detailed description of internationally accepted types of prisons are described in (Table 2.2).

Table 2.2: Prison categories in the United Kingdom and Wales.

Category	**Prison Type**	**Description of Prison**
A	Closed Prison	Prisoners whose escaped would be extremely dangerous to the public or national security eg. Those arrested for supplying of explosives, attempted murder, robbery, supplying of Class A drugs, rape etc.
B		Prisoners who do not need maximum security yet their escape needs to be made very difficult.
C		Prisoners who cannot be trusted in open conditions yet they are not likely to try to escape
D	Open Prison	They are rationally trusted not to try to escape. Such prisoners are given release on temporal licence (ROTL) to work in the community or go no home leave once they have passed their full licence eligibility dates (FLED) which is usually a quarter of the way through their sentence.

Category A prisoners are further divided into Standard Risk (Minimum), High Risk (Medium), and Exceptional Risk (Maximum), based on their likelihood of escaping ("Prison Service Instructions 2010," n.d.). In maximum prisons, movements and activities of prisoners are authorized and monitored. Movements and activities of prisoners in medium prisons are subject to locally specified limited restriction and supervision and in minimum prisons all activities and

movements, specified locally, are subject to minimum supervision and restrictions. Prisoners in minimum prisons may be entitled to release on temporary licence and their activities in the community are not monitored.

The Ghana prison service comprise of 45 institutions which include the prison headquarters, prison officers training school, senior correctional Centre formally known as Ghana Borstal Institute, 14 local prisons, 9 agricultural settlement camp prisons, 7 female prisons, 7 central prisons, 1 maximum security prison, 1 medium security prison, and 3 open camp prisons. The prisons have a sanctioned holding knack of 9,875 prisoners (Ghana Prisons Service, 2014).

Owing to inadequate facilities, the Ghana Prison Service is compelled to further classify the prisons based on activities undertaken at the various prison establishments. Based on this form of classification there are four categories of prison in Ghana;

Central Prisons: In this prison setting, trade training facilities are provided to arm inmates with employable skills for so that they can be useful to themselves and humanity. Central prisons secures long sentenced prisoners. It is the pivotal point for every category of prisoners with exception of prisoners that have been condemned. Central prisons are also known as minimum security facility. Owing to limited facilities, these prisons facilities perform same functions as the medium security by taking custody of high sentence prisoners (Ghana Prisons Service).

Local Prisons: This prisons give attention to the safe keeping and wellbeing of inmates as a result of insufficient space for trade training activities. It houses mostly short sentenced prisoners. This facility is also known as minimal internal security fortification.

Open Camp Prisons: This prisons are agriculturally oriented and embark on agricultural activities that provide food and equip inmates in contemporary agricultural practices. Inmates who are

about to go on discharge are usually conveyed to these facilities and prepared for the outside world.

Agricultural Settlement Camps: Security arrangements in this prisons are very relaxed and the prison is usually not fenced. The key objective is to train prisoners who will soon be discharged in agricultural activities that will produce enough food to supplement the feeding of inmates and generate some income for the Service.

The Maximum Security prison of Ghana is a highly fortified prison facility designed to hold the most aggressive and incorrigible inmates. It was built to meet international maximum-security specifications and standards and has a ratified capacity of 2000 prisoners, it has a state of the art health Centre, a versatile court, a football park, inmates' dormitories, and an administrative block. The facility also has a kitchen, a corn mill house, admission and discharge block, visitors' lounge, a gate lodge, a standby generator and a bio gas plant among others. There is strict control and monitoring of inmates' movement. Interaction between inmates and staff is very minimal and there is a 24-hour surveillance of inmates and the entire facility.

The Maximum Security facility comes close to the maximum but with little stress on internal fortification. This type of prison was used to hold high sentence and aggressive prisoner until the provision of a maximum prison facility in 2011. Till date there is only one of such facility in the Ghana and it is located at Nsawam in the Eastern Region.

As at the year 2014, the Ghana Prison Service had a total inmate population of 13,164 as against the authorized holding capacity of 9,875 resulting in a 33.31% overcrowding. Out of the total inmate population, 13,005 are males and 159 are females. The total number of convicted prisoners was 11, 301 and the remaining 1,863 were unconvicted. Majority of convict peisoners

admitted in 2014 fall within the age of 18 – 25 (41.52%) followed by 26 – 35 (28.85%), and then 36 – 45 (15.74%) with the least population being people aged 60 and above (2.05).

2.5 TRANSMISSION OF HBV IN CORRECTIONAL FACILITIES

The challenges presented by overcrowded prisons around the globe frequently leash other grave problems. Prisons that are overcrowded are more likely to be difficult to control, fierce, and unhealthy (SADC, 2009). Among the high risk populations, individuals imprisoned in correctional systems endure a larger encumbrance of infectious diseases, including hepatitis B virus (HBV) infection (EMI, 2016). Since HBV is a blood born infection linked with sharing of contaminated needles and other sharp instruments as well as tattooing, it is of great importance in the prison setting where such activities prevail (SADC, 2009). In as much as risk behaviors that can transmit HBV infection are often prohibited at correctional facilities, inmates yet engage themselves in such activities. A study conducted by Khan *et al*. (2005) at a Georgian state correctional facility reported a high prevalence of HBV infection and a high rate of ongoing HBV transmission within the facility. In all the cases, only a few of the inmates had clinical symptoms of acute hepatitis B and the rest were identified through serological testing. Similar observations were made in other parts of the world with Ghana not being an exception (Adjei *et al*., 2006). Yearly, inmates are released to the community or relocated to another correctional facility, thus serving as a reservoir for ongoing transmission of HBV. Clustering of unique HBV nucleotide sequences among inmates during the study provided a strong evidence of chains of transmission between inmates. Living in close proximity to one another and overcrowding is a predisposing factor which promotes all sorts of activities that could pose risk of HBV infection

to inmates. Unfortunately this is what prevails in our prisons, thereby serving as a good medium for transmission of many unfavorable health conditions.

According to CDC (2015), majority of inmates with identified acute HBV infections after serologic testing in United States of America (USA) were housed in multiple Georgia correctional facilities and were infected at some stage of their imprisonment. This suggests constant transmission in multiple correlational facilities. Although there is insufficient data on the general burden of HBV infection in correctional systems, the ongoing transmission demonstrated in Georgia prisons has also been demonstrated in Ghana and some other states where similar conditions prevail. Ghana's data indicate HBsAg prevalence of 17.2% in prison inmates than in the general population, suggesting probable transmission through intravenous drug use, unsafe sexual behaviour and tattooing as pertains to prisons worldwide (Dolan and Larney, (2010); Adjei *et al*., (2006); Khan *et al*., (2005). In a quest to determine the prevalence and determinants of Hepatitis B antigenemia in 15 007 Inmates in Taiwan, a history of blood transfusion due to injuries from criminal actions was also reported to be a predisposing factor for horizontal transmission of HBV (Lin *et al*., 2010).

Complete surveillance of infectious diseases such as HBV, HIV, HCV and syphilis in the incarcerated population in Ghana is wholly absent, and none of the 46 prisons in Ghana have a program for routine HBV immunization and treatment (Adjei, 2006). Conditions prevailing in Ghanaian prisons are poor with overcrowding, poor nutrition and inadequate healthcare. Reports also indicate that a great deal of unsafe sexual activity, drug injection and needle sharing occurs among prisoners in Ghana thereby contributing to HBV transmission in prisons (Adjei, 2006).

2.6 HBV GENOTYPE DISTRIBUTION

The deviations in the sequence of the whole genome of HBV (>8 %) has resulted in the grouping of the genotypes into ten assigned as A to J (Chook *et al*., 2015; Spitz, Mello, & Araujo, 2015; Zhang *et al*., 2007). HBV genotypes have discrete topographical distributions and the worldwide spread of HBV genotypes may be associated with tribal differences, method of transmission, environmental factors or all of these elements (Zampino *et al*., 2015b). Genotype A is largely dispersed universally and is the principal genotype in Africa, Europe, North America and India, while genotypes B and C overshadow the other genotypes in East and Southeast Asia (Norder *et al*., 1993; Kao and Chen 2006). The predominant genotype in the Middle East and Mediterranean county and India is the genotype D, and genotype E is mostly located in Sub-Saharan Africa. (Mulders *et al*., 2004; Kramvis *et al*., 2005; Singh *et al*., 2009). In America, Mexico, France and Germany genotype G is the trending genotype (Tanwar and Dusheiko 2012). Genotype G usually co- exists with other HBV genotypes more especially genotype A. The latest genotype was detected in Vietnam, China and Laos is the genotype I (Phung *et al*., 2010; Olinger *et al*., 2008), and the latest genotype; J was identified in the Ryukyu Islands in Japan (Tatematsu *et al*., 2009). Genotype A, B, C, D and E are often discovered in Africa and the trend of circulation is progressively materializing. For instance genotype A outweighs the other genotypes in southern and Eastern Africa, genotype E dominates the vast region from Senegal to Namibia and eastward to the Central African Republic while genotype D prevails in northern Africa and . Genotype E is also often uncovered in the Central African Republic, Benin, Togo, Nigeria and the Democratic Republic of the Congo (Zampino *et al*., 2015a). An A/E and A/D recombinant genotypes of HBV have also been discovered in Ghana, Western Africa, Cameroon and still counting in healthy black African adults (Zampino *et al*., 2015; Ayiku, 2015;

Garmiri *et al*., 2009; Candotti, Danso, & Allain, 2007). In Ghana the predominant genotype is the genotype E (Dongdem *et al*., 2016; Geretti *et al*., 2010; Garmiri *et al*., 2009; Zahn *et al*., 2008; Candotti, Danso, & Allain, 2007). The inherent variability of HBV presents a challenge for the sensitivity of immunologic and molecular based assays. Some genotypes have been reported to predispose the patients to some adverse clinical outcomes of the HBV infection (Guettouche & Hnatyszyn, 2005).

2.7 MUTATIONS IN THE GENOME OF HEPATITIS B VIRUS

Mutations do arise in the genome of HBV due to the error prone nature of reverse transcriptase during the reverse transcription phases in HBV replication. The host immune system is also one of the many factors that largely affect the natural course of HBV and leads to mutations in the genomic structure (Balistreri, 2005). Scrutiny of HBV whole genome has offered pertinent pieces of information on mutations that occur across all the four open reading frames.

2.7.1 PRECORE (PC) MUTANTS

The initial immune escape mutants of HBV revealed is the mutation in the precore region of the genome and according to Wai & Fontana, (2004) these HBV variants emerge during HBeAg seroconversion. Mutations in the PC region prevent the production of HBeAg, even though there is continuous formation of infectious virions. Frequently observed mutation is a G to A substitution at nucleotide position 1896. This substitution averts the production of HBeAg by initiating a premature stop codon into the open reading frame of the precore domain thereby truncating the precore/core protein into a 28-amino acid peptide (Suppiah *et al*. 2014; Tong et al. 2005; Tacke *et al*., 2004). Since the precore region is not vital for HBV replication or hepatitis B core antigen (HBcAg) expression, the G1896A variant is replication-competent and is infectious.

G1896A mutation appears only in some particular viral genotypes example B, C, D, and E (Tong *et al*., 2005);. PC mutation G1896A + C1858T creates a translational stop codon resulting in absent HBeAg expression (Suppiah *et al*., 2014; Tacke *et al*., 2004). Possibility of precore escape mutants should be cogitated in persons who display HBeAg negativity, HBsAg positivity, anti-HBe positivity, HBV DNA positivity, and elevated serum aminotransferase levels. Conversely the precore mutant is not evenly pathogenic such other contributing factors such as co-mutations or host factors probably play a part in the more virulent forms of precore mutant associated disease (Suppiah *et al*., 2014).

2.7.2 CORE PROMOTER MUTANTS

Another usual HBeAg variant is the core promoter mutant, depicted by point mutations in the promoter for both HBeAg mRNA and core protein mRNA (Tong *et al*., 2005). The most common mutations in the core promoter are A1762T and G1764A occurring in tandem (Suppiah *et al*., 2014; Garmiri *et al*., 2009). Mutations have also been detected at nearby positions such as 1753, 1757, 1766, and 1768 and a site-directed mutagenesis experiments proposed that supplementary mutations are likely to occur at positions 1753, 1766, and 1788 (Tsai *et al*., 2009). This causes further decline in HBeAg expression and enhanced genome replication. A1762T/G1764A/C1766T triple mutant has also been reported and assumed to have a greater than 10 fold higher replication capacity than the wild-type virus (Tsai *et al*., 2009). The measure of changes in HBeAg expression and genome replication correlates with a reduction in precore RNA and an increase in pgRNA levels. Consequently, growing numbers of core promoter mutations may perhaps activate up regulation of genome replication and down regulation of HBeAg expression (Tsai *et al*., 2009).

Studies have shown that certain genotypes are more prone to core promoter mutants than other genotypes. Yuan, Zhou & Tanaka (2007), in their work on hepatitis B virus mutations in core promoter and precore gene and their clinical implications reported a higher prevalence of A1762T/G1764A double-mutation in genotype C1 (with C1858) than C2 (with T1858). Genetic and serological classification of hepatitis B virus genotype A and D infected blood donors in Poland also established higher prevalence of Genotype A core promoter mutations than genotype D. Tong *et al*. (2005) and Tacke *et al*. (2004) also reported the double A1762T and G1764A nucleotide exchange and accentuated that this leads to a considerable reduction in HBeAg expression but heightened viral genome replication. According to Tong *et al*. (2005) and Tacke *et al*. (2004), decrease in HBeAg expression is actually intervened by decrease precore RNA transcription, while the machinery for heightened replication is unclear. These properties of the HBV lead to enhanced pathogenicity of core promoter mutants in vivo. The excellent replication ability and lowered virion secretion could increase viral load in the liver, which would call for mounting of an immune response thereby leading to damage of the liver. The incidence of core promoter variant is about 40% and it is uniformly dispersed amongst the HBV genotypes. Fulminant types of HBV associated hepatitis have been connected to infection with core promoter mutants however further studies are needed to establish the association (Tong *et al*., 2005; Tacke *et al*., 2004). Suppiah *et al*. (2014) also reported the following basal core promoter mutations; A1762T-G1764A double mutation, C1653T, A1752G and C1766T.

2.7.3 X GENE MUTANTS

Among all the HBV genes the HBV X gene is the smallest and it expresses a 154-amino-acid multipurpose protein called HBx, with an N-terminal negative regulatory/antiapoptotic domain

and a C-terminal transactivation/proapoptotic domain (Lee *et al*., 2011). According to Lee *et al*. (2011) the inexplicable X gene seems to perform an array of function at the cell level that can possibly lead to the development of hepatocellular carcinoma (HCC). Contradictory suggestions exist on the functional bustle of HBx, while some have reported that it stimulate the development of HCC others debated that fact. HBV core promoter overlaps partially with HBx coding sequence, so the nucleotide 1762 and 1764 mutations induce HBV X protein (HBx) 130 and 131 substitutions (Lee *et al*. 2011).

Direct sequencing by Lee *et al*. (2011) showed the following X gene amino acid mutations; HBx131, HBx130, HBx5, HBx94, and HBx38 in hepatocellular carcinoma (HCC) patients. Double mutation such as HBx130 + HBx131 and triple mutation, HBx5 + HBx130 + HBx131 were significantly high in HCC patients and double and triple mutations increased the risk for HCC by 3.75-fold (95% confidence interval [CI] = 1.101 to 12.768, P = 0.033) and 5.34-fold (95% CI = 1.65 to 17.309, P = 0.005), respectively, when HCC patients were compared to Chronic Hepatitis B (CHB) patients. Since double mutations in the BCP region also exist in the coding sequence of HBV X gene, they convert K to M at position 130 and V to I at position 131 in the overlapping X-open reading frame gene product and to date, these double mutations have been suggested to arrest the transcription of the precore RNA but without a serious impact on the transcription of the pregenomic RNA. Garmiri *et al*. (2009) also reported the hepatitis b virus x gene mutation at amino acid position, 94, 130, 131 where and Y substituted an H, M substituted a K and an L substituted a V respectively. Two of the sequences had coexisting substitutions of H94Y and K130M and seven other sequences had K130M coexisting with V131I (Garmiri *et al*., 2009).

2.7.4 S GENE MUTANTS

The S gene of the hepatitis B virus genome codes for surface envelope proteins and is accountable for cataloging of HBV strains. The pre-S regions play an indispensable role in the interaction with the immune responses because they comprise several epitopes for T or B cells, thus a deletion in this region appears to yield escape mutants that elude host immune surveillance (Shi, *et al*., 2012). Neutralizing antibodies (anti-HBs) target the "a" determinant and mostly lead to clearance of HBV. The antigenic structure of the virus is nonetheless transformed as a result of mutation caused by active or passive immunization and this results in the development of escape mutants. In HBV DNA positive children from four sequential surveys in Taiwan, the prevalence of hepatitis B surface gene 'a' determinant mutants increased from 7.8% before the launch of a vaccination program, to 19.6% at 5 years after, 28.1% at 10 years after, and 23.1% at 15 years after the program (Mei-Hwei *et al*., 2006). Most mutations occur in the MHR of the S gene and these mutations create a cause for alarm as they can lead to reactivation of hepatitis B and occult hepatitis B infection (Shi, *et al*., 2012). The most commonly reported HBsAg mutant is G145R and it demonstrates the highest occurrence in both vaccinated and random populations (Suppiah *et al*., 2014; Shi *et al*., 2012; Velu *et al*., 2008). Mutational analysis in the S gene of genotype C patients demonstrated two significant mutations which were W182 stop codon and deletion at open reading frame (ORF) of pre-S1 with the frequency occurrence of 2.2% (2/93) and 5.4% (5/93), respectively (c). The existence of W182 has been linked with premature termination in the pre-S1 region which eventually averts the expression of S gene full length. Correlation between HBV genotypes and incidence of W182 mutant is not plainly understood however Suppiah *et al*. (2014) proposed that it may be associated with genotype C and that it could serve as a molecular marker to predict HCC.

Other S gene mutations reported by Shi *et al*. (2012) include; T/I126S, Q129H, G130N, S143L, D144A, G145A, and G145R. In a study that attempted to establish intra-familial horizontal transmission of hepatitis B virus surface antigen mutant, serum samples from family members of 10 vaccinated infants were collected and analyzed by polymerase chain reaction (PCR) and sequencing. G145R mutant was identified in family members of three of the 10 infants used in the study (Oon , Chen, Goo & Goh, 2000). The G145R mutant was present in samples with high levels of neutralizing antibody against HBsAg (anti-HBs). In addition to G145R mutant, a closer examination of the nucleotide sequence data revealed changes in other codons such as codon 130, 131, 146, 154 and codon 155. In one of the families studied, the fathers sample was found to contain G145R coexisting with the WT virus and a Y161F mutant while the WT virus was found in the mother's sample. This indicated clearly that within a family, it is possible for one individual to horizontally transmit HBV mutants such as G145R to another (Oon , Chen, Goo & Goh, 2000). Mei-Hwei *et al*. (2006) in their study on Hepatitis B Virus Mutation in Children, found a surface gene mutant (residue 129, Gln to Arg) in two mothers and their newborn evoking a direct mother to child transmission. Sequence analysis of the S-gene by Thakur *et al*., (2005) revealed mutations at amino acid T118V (up-stream flanking region of the ''a'' determinant) and amino acid A128V. S117R, T118V and other mutations in the ''a'' determinant such as T126S, A128V, K141D, P142R, T143M, G145R and two other nonsense mutations (C137Stop and C138Stop). A T123A mutant was also reported to have escaped assay detection (Geretti *et al*., 2010).

2.7.5 POLYMERASE GENE MUTATIONS

HBV surface gene is overlapped completely by the polymerase gene as a result of these mutations that arise in the S gene may also lead to changes in the polymerase gene. Interferon and nucleoside or nucleotide analogs (NA) have long been used for management of HBV infection. NA suppress replication of HBV by contending for incorporation into viral DNA and has greatly reduced the dangerous consequence of HBV infection even with poor off-treatment responses and failure of cccDNA abolishing (Liu *et al*., 2014). Extended use of NA's in the treatment of HBV infections has amplified the risk of developing drug resistance, which possibly will lead to treatment failure. Because of this several types of drug resistance mutations have been detected in HBsAg positive individuals (Table 2.3). Lamivudine is the extensively used NA and can be used as single or in

combination with other NAs nevertheless, the drawbacks are palpable: it is inclined to select drug resistant mutation with the highest resistance rate for all known NAs (Liu *et al.*, 2014).

Table 2.3: Polymerase Gene Mutations and Association with Drug Resistance

SITE OF MUTATION	DRUG	SOURCE
M204I M204V M204I/V V173L L180M M184V I195M M204Q V173 + L180M + M204V M204I L180M+M204V	Lamivudine	(Utsumi & Lusida, 2015; Liu *et al*., 2014;Shi *et al*., 2012);Lok *et al*., 2007; Zoulim & Fournier, 2007; Tacke *et al*., 2004;Delaney *et al*., 2003).
M204I and same drug resistance mutants as lamivudine	Telbivudine	(Lok *et al*., 2007;Lai *et al*., 2005)
A194T	Tenofovir	(Pastor, 2009)
A181V/T L217R N236T	Adefovir	(Ismail *et al*., 2012) (Bottecchia *et al*., 2008).
V214A N238T	Adefovir	(Pastor 2009)
Combination of M204V/I, L180M and one of the following mutations – M184S/C/G/A, rtS202G/I/C, or rtM250V	Entecavir	(Zhao *et al*., 2016)

2.8 HORIZONTAL TRANSMISSION OF HBV MUTANT STRAINS

HBV surface antigen mutations specifically those that occur within "a" determinant may cause a conformational change in the antigenic region of the protein thereby resulting in the flop of host antibodies to bind to the antigen and neutralize its effect. This results in the escape of this antigen from the host immune response and active replication of the virus within the liver (Moradi *et al*. 2012). A greater number of mutations that occur in the major hydrophilic region are associated to immunotherapy and vaccine escape as well as failure of HBsAg detection (Moradi *et al*. 2012). An evidence of horizontal transmission was seen in a study by Chen, Oon & Koh (2000) where a child born to an HBsAg positive mother still tested positive for HBsAg after six years of age even after receiving his vaccine shots as recommended. After direct sequencing and analysis Asp144Ala mutant was detected in the child's serum and this was later seen in the S gene sequence of the mother who initially haboured the wild type HBV. In a subsequent study Thakur *et al*. (2005) also reported the possible horizontal transmission of G145R mutant HBV to related and unrelated contacts after surface gene sequencing including the "a" determinant region was done for the positive samples. Sequences were aligned and compared for homology. Three household contacts were also identified with mutation G145R along with T118V and T143M. The appearance of these mutations in the household contacts provides an indirect evidence for the possible horizontal transmission of G145R variant to a both related and unrelated contact (Thakur *et al*., 2005).

CHAPTER THREE

METHODOLOGY

3.0 INTRODUCTION

This study was derived from a cross-sectional nationwide survey performed in all correctional facilities across the country. It is also a follow-up of a study which showed characteristic mutations across the s-gene of HBV strains found in prison inmates that formed the population used in this study (unpublished).

3.1 STUDY DESIGN AND POPULATION

As part of a national study on prevalence of HBV and HIV in prison facilities in Ghana, plasma samples from inmates from prisons across the country were achieved with the consent of study participants. A preliminary study of a particular central prison using the s-gene has shown a unique mutational pattern and some evidence of transmission. From a cross-section of inmates in this central prison, samples from 24 inmates who were tested and confirmed as infected with HBV was used for this study. Two other plasma samples from 2 inmates from other prisons whose S gene sequence had suggested a close relationship with S gene sequences form the study prison were included in the genetic analysis for quality assurance.

Data such as duration of incarceration, nature of the prison environment and risk behaviours were obtained through a standardized questionnaire used during the national survey.

3.2 SAMPLE SIZE AND COLLECTION

All the 26 HBsAg positive archived samples were previously screened for anti-HBe IgM and HBeAg. Out of the 24 samples from the same prison, 12 were positive for HBeAg and 2 were positive for anti-HBc IgM. The 2 samples from the other prisons were positive for both HBeAg

and anti-HBc IgM and were identified by the Identity number (ID) 2I and 9I. In total out of 26 samples, 14 were HBeAg positive and out of the 4 were positive for anti-HBc IgM. The correctional facility with 24 samples was nominated because anti-HBc IgM and HbeAg are indicators used to establish the level of recent infections and transmissibility respectively, thus a correctional facility with comparatively high incidence of both markers was ideal for genetic investigation to validate intra-prison transmission. The 2 other samples were also used because of how closely related their S gene sequences were and considering the fact that they were incarcerated in the same prison at a point in time which could mean that one inmate infected the other. Samples from inmates with anti-HBc IgM were identified with "I" attached to a number.

Achieved plasma samples in a study bank were used for the study. The inmates signed a consent and specimen banking forms during the enrolment (Refer to Appendix II).

3.3 LABORATORY ANALYSIS

3.3.1 HBV DNA EXTRACTION

HBV DNA was extracted from all the 26 HBsAg positive archived plasma samples using the Zymo Research Viral DNA/RNA kit. The extraction process was achieved by tailing the manufacturer's instructions. Five hundred microliters (500µl) of viral DNA/RNA buffer was pipetted into an eppendorf tube and two hundred and fifty microliters (250µl) of plasma was added and mixed by inverting it every five minutes for 30 minutes. The samples were transferred into a Zymo- spin IIC-XL column in a collection tubes and centrifuged at 7000xg for 4 minutes followed by a quick spin. The flow through was discarded and Five hundred microliters (500µl) of viral wash buffer was added to the column and centrifuged at 12000xg for 2 minutes The collection tubes were discarded and the column was carefully transferred into a DNase/RNase –

Free tube. 35µl of DNase/RNase – Free buffer was added directly to the column and kept for a while to help the column to properly soak the buffer. It was centrifuged at 18000xg for 1 minute to obtain the DNA. The column was discarded and the DNA in the tube was stored at -20°C until needed and used as a template for polymerase chain reaction (PCR).

3.3.2 AMPLIFICATION OF HBV GENOMIC REGIONS

The S gene, PreS1/PreS2 gene and the Precore/Core gene of HBV for all the samples (n=26) which includes fourteen HBeAg positives and four anti-HBc IgM indicating new infections were amplified.were amplified by means of a Nested Polymerase Chain Reaction (PCR). Primers P1WR5-F and P5W-R were used for the first round PCR and primers in table 5 were used for the second round PCR The LongAmp® *Taq* 2X Master Mix kit (LongAmp® *Taq* 2X Master Mix New England Biolabs Inc, 2016) was used for the PCR and the manufacturer's protocol was carefully followed.

3.3.2.1 FIRST ROUND PCR AMPLIFICATION

For the first round PCR amplification, the total volume of the Master Mix for 26 reactions was 936 microliters (µl) and consisted of the following in their assorted volumes per reaction; Long Amp Taq 2* Master Mix (468 µl), P1WR5-F and P5W-R primers (23.4µl each), nuclease free H2O (89.7µl), MgCl2 (19.5µl). Twenty four microliters of the master mix was aliquoted into PCR tubes and 12µl DNA template was added to each tube making a total volume of 36 µl for each reaction. PCR was done using specific primers for HBV whole genome sequences. The thermocycling conditions for the PCR were as follows; there was an initial denaturation step of 2 min at 94˚C, followed by 20 cycles of denaturation at 94˚C for 30 sec, annealing at 47˚C for

1min and primer extension at 65˚C for 7 min, followed by a final primer extension at 65˚C for 15 min.

3.3.2.2 SECOND ROUND PCR AMPLIFCATION

The second round PCR assay mixture targeted HBV Precore/Core gene, PreS1/PreS2 gene as two overlapping fragments: fragment A (1014 bp), and fragment B (1074 bp) designated as S1 and S2 respectively (figure 3.1). This was achieved by using an established protocol by Zhang *et al*., (2007) with slight modifications. The S gene was also targeted in this round of PCR using a different set of primer (HBV-F/ HBV-R).

Table 3.1: Primers used for first round whole genome PCR

Name	**Primer Sequence**	**Length**
P1WR5 (Sense)	5'- TTTTTCACCTCTGCCTAATCA- 3'	21
P5W (anti - sense)	3'- AAAAAGTTGCATGRTGMTGG- 5'	20

Table 3.2: Primers used to amplify the Precore/Core gene and PreS1/PreS2 region in the second round PCR

Name	Primer Sequence	Length	Location	Amplicon size (bp)
P1 (FA1-L) (Sense)	TTTCACCTCTGCCTAATCATCTC	23	4	1014
P2 (FA1-R) (anti-sense)	TCTTGTTCCCAAGAATATGGTG	22	1018	
P1 (FA2-L) (Sense)	GCGTCGCAGAAGATCTCAAT	20	593	1074
P2 (FA2-R) (anti-sense)	TTGAGAGAAGTCCACCACGAG	21	1667	

FA1-L/ FA1-R were the primers used for fragment A (S1) and FA2-L/FA2-R were the primers for fragment B (S2) (Zhang *et al*., (2007).

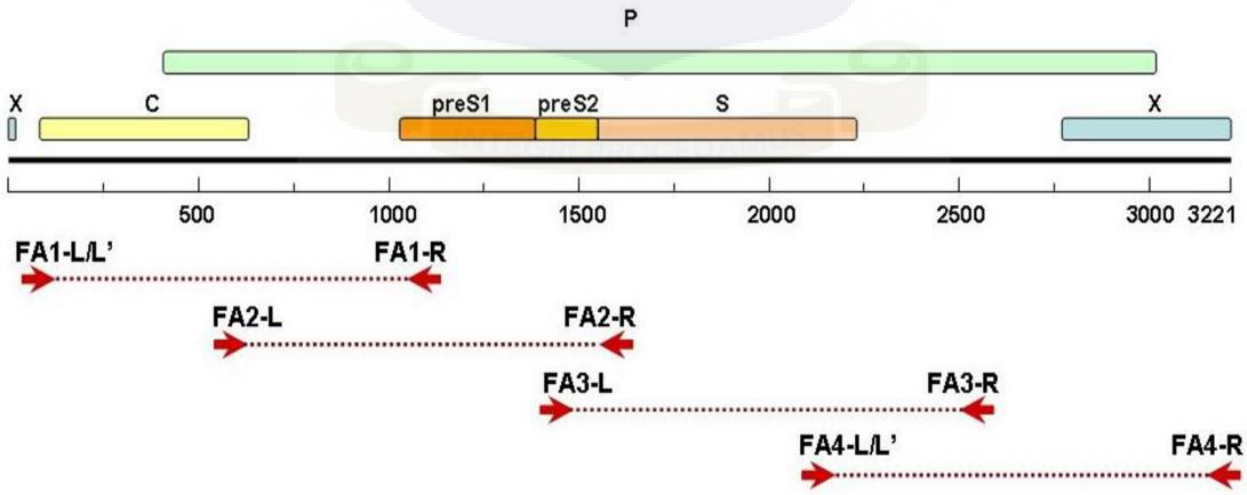

Figure 3.1: Illustration of open reading frames of HBV and designed primers used (Zhang *et al*., 2007).

3.3.2.3 AMPLIFICATION OF PRECORE/CORE GENE AND PRES1/PRES2

For the amplification of the Precore/Core and Pre S1/Pre S2 genes, the total volume of the Master Mix for 26 reactions for each of each fragment (S1 and S2) was 936 microliters (µl) and consisted of the following in their assorted volumes per reaction; Long Amp Taq 2* Master Mix (468 µl), P1-F and P2-R primers (23.4µl each), nuclease free H2O (89.7µl), MgCl2 (19.5µl). Twenty four microliters of the master mix was aliquoted into PCR tubes and 12µl of 1:125 dilution of the first round product served as the template making a total volume of 36 µl for each reaction. The thermocycling conditions for the PCR were as follows; there was an initial denaturation step of 2 min at 94˚C, followed by 45 cycles of denaturation at 94˚C for 30 sec, annealing at 52˚C for 1min and primer extension at 65˚C for 2 min, followed by a final primer extension at 65˚C for 10 min. The PCR products were visualized on a 2% ethidium bromide-stained agarose gel. PCR products were processed and sent for commercial sequencing.

The surface gene (500bp) was amplified for all the samples. A published protocol by Gous (2006) was used with some slight modifications. DNA samples were amplified using the Forward Primer. (HBV F):.5’ GTGGTGGACTTCTCTCAATTTTC-3’and was between map positions 256 to 278 of the HBV genome. Reverse Primer (HBV R): 3’-CGGTATAAAGGGACTCACGAT-5’ was also mapped between positions 796 to 776. The total volume of the Master Mix for 26 reactions was 936 microliters (µl) and consisted of the following in their assorted volumes per reaction; Long Amp Taq 2* Master Mix (468 µl), P1-F and P2-R primers (23.4µl each), nuclease free H2O (89.7µl), MgCl2 (19.5µl). Twenty four microliters of the master mix was aliquoted into PCR tubes and 12µl DNA template was added to each tube making a total volume of 36 µl for each reaction. Primer P1 was reconstituted by adding 511.18 µl of nuclease free water to a 100 µM Stock solution. Primer P2 was also

reconstituted by adding 192.15 µl of nuclease free water to a 100 µM Stock solution. A previously amplified HBV DNA-positive patient serum sample served as a positive control. The thermocycling conditions for the PCR were as follows; initial denaturation step was for 5 min at 94˚C, followed by 42 cycles of denaturation at 94˚C for 30 sec, annealing at 50˚C for 1 min and primer extension at 65˚C for 1min, followed by a final primer extension at 65˚C for 10 min.

3.3.2.4 AGAROSE GEL ELECTROPHORESIS OF PCR PRODUCTS

The final product of the PCR was run on 2% agarose gel. The gel was produced by dissolving 2 g of the agarose powder into 100 ml of 1x Tris Acetate EDTA (TAE) buffer in a Pyrex bottle. The suspension was heated in a microwave till a uniform solution was attained. The solution was allowed to cool and 2 µl of ethidium bromide was added. This was swirl and quickly poured into a gel cast system with combs to form the needed wells. The gel was allowed to solidify and then tran5ferred into a gel tank filled with TAE buffer. Five microliters of the PCR product was loaded into the wells and allowed to run at a voltage of 100v for 1 hour. The gel was observed and photographed over a UV transilluminator to identify any bands corresponding to various HBV gene segments (overlap). The sizes of the bands (PCR products) where estimated by comparing with the mobility of a standard 1kb DNA ladder (7 µl).

3.4 NUCLEOTIDE SEQUENCE AND AMINO ACID ANALYSIS

PCR products were sequenced commercially by INQABA BIOTEC, South Africa. The sequences were subjected to the Basic Local Alignment Sequencing Tool (BLAST) inbuilt in the CLC Work Bench version 7. The evolutionary history was inferred using the Neighbor-Joining method (Saitou. and Nei 1987) in built in the Molecular Evolutionary Genetics Analysis software

version 7. The bootstrap consensus tree was inferred from 1000 replicates taken to represent the evolutionary history of the taxa that was analyzed (Felsenstein, 1985). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was showed next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, Nei & Kumar 2004; Kumar, Stecher & Tumura 2015) and were in the units of the number of base substitutions per site. Sequences were aligned to well-known sequences and all positions containing gaps and missing data were eliminated. HBV genotypes were confirmed using the Stanford Database online tool and jumping Profile Hidden Markov Model Tool. (https://hivdb.stanford.edu/HBV/HBVseq/development/HBVseq.html.and.https://jphmm.gobics.de/submission respectively)

3.5 DATA MANAGEMENT AND STATISTICAL ANALYSIS

The sequences were aligned to known reference sequences and edited using CLC workbench 10.1, and BioEdit version 7.2.5. Similarity Index Matrix was done using BioEdit 7.2.5 Mutational analysis was done using the online tool Geno2pheno (http://hbv.bioinf.mpi-inf.mpg.de/) and phylogenetic analysis was carried out using Molecular Evolutionary Genetics Analysis software (MEGA) 7.0.14.

3.6 ETHICAL ISSUES

All participants consented to participate in specimen banking which permits further studies to be done on their samples (Refer to Appendix II). The study was approved by the Ethical and Protocol Review Committee of the University of Ghana School of Biomedical and Allied Health Sciences, College of Health Sciences.

CHAPTER FOUR

RESULTS

4.0 INTRODUCTION

Over all the S gene was successfully amplified for all the 26 samples, 25 out of the 26 had a successful amplification of the Precore/Core gene and 15 out of the 26 samples had their PreS1/PreS2 genes successfully amplified. However, 17 out of the 26 samples were successfully sequenced for S gene analysis, 8 out of the 17 was sequenced for precore core gene analysis and 5 out of the 8 samples were successfully sequenced for the PreS1/PreS2 gene analysis.

4.1 DEMOGRAPHIC AND BEHAVIOURAL RISK DATA

Demographic data for all the 17 inmates were obtained (Table 4.1). All the 17 inmates tested positive for HBsAg marker and 4 of them were ant- HBcIgM positive. Eight of this inmates have been in this particular prison 0 - 6 months, 4 of them have been in prison for 7 – 12 months and 3 of the inmates have spent over the 2 years in this prison. One inmate had a history of being transferred from one prison to another.

Behavioural risk data was collected on this inmates (Table 4.2). Ten inmates had a history of having blood contact within prison. Five out of this 10 respondent had a history of blade sharing whiles in prison, 3 had a tattoo and 1 inmate reported that he used the same tattoo piercing instrument (TI) with others. One of the inmates had a blood covenant within prison (WP). All the 17 inmates were narcotic drug users and 2 of them is still was still on drugs in prison at the time of data collection. None of the inmates injected drugs. Data on sexual contact as a risk factor was assessed in this inmates. Ten of them had sexual partners before being incarcerated and 2 of

these inmates had also been involved with commercial sex workers. Twelve out of the 17 inmates reported that they have been forced to have sex with other inmates within the prison.

Eight inmates reported that they had never used a condom during sexual intercourse and 3 inmates admitted that they use condom either a few times during sex.

Table 4.1: Summary of Demographic and Serological Data of inmates

SUMMARY	**n=17**	**Years in this prison**	**n=17**
Total	17	0 - 6 months	7
HBsAg	17	7 - 12	6
HBeAg	13	> 2	4
IgM	4		

Inmate with identification number 2I (8) had a transfer history

Table 4.2: Summary of Behaviour Risk Data of inmates whose samples were successfully amplified and sequenced

BLOOD CONTACT (n = 10)	
Blade Sharing	5
Tattoo	3
Same TI used WP	1
Blood Covenant WP	1
INJECTING DRUG USE (n = 10)	
Drug Users	10
Injected Drug	0
Share needle	0
SEXUAL CONTACT (n=34)	
Casual Sex Partner OP	10
1 Partner	8
2 Partners	1
4 Partners	1
Commercial Sex Workers OP	2
Force Sex WP	12
CONDOM USE (n = 11)	
Every Time	1
Most Times	1
A few Times	1
Never	8

TI, Tattoo piecing implement; WP, within prison; OP, outside prison; n, number of respondents.

4.2 HBV SURFACE GENE AMPLIFICATION

The S gene of all the 26 samples were successfully amplified. However 9 samples showed very faint bands thereby disqualifying them for sequencing. Expected band sizes were seen on a 2% Agarose gel (Figure 4.1).

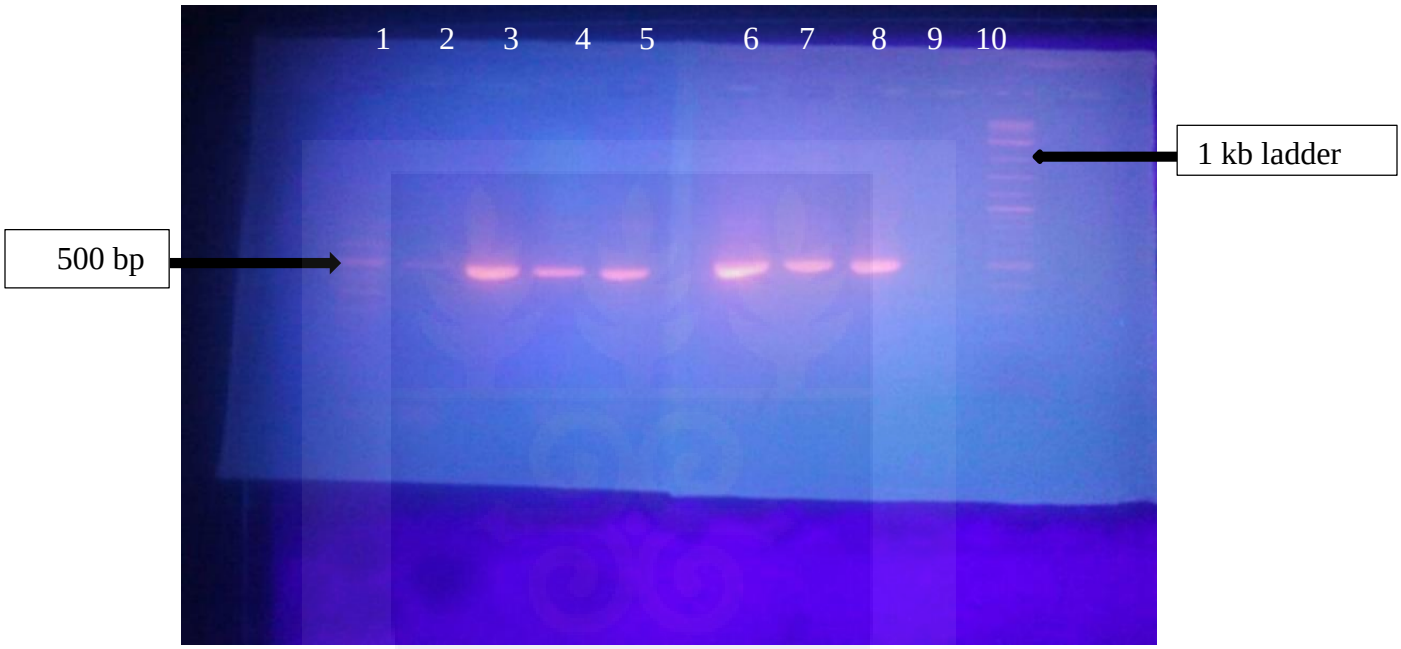

Figure 4.1: A representation of a 2% Ethidium bromide-stained Agarose gel electrophoresis of S-gene specific PCR amplicons. Lanes 1 & 10 – 1 kbp molecular ladder; 2-7 shows successfully amplified bands (500bp). Lane 8- Positive Control (PC); Lane 9- Negative Control (NC).

4.3 HBV PRECORE/ CORE GENE AMPLIFICATION (S1)

This region was successfully amplified in 25 out of the 26 samples used for this study. Out of these 25 only 8 showed very good bands (Figure 4.2) and were sent for sequencing.

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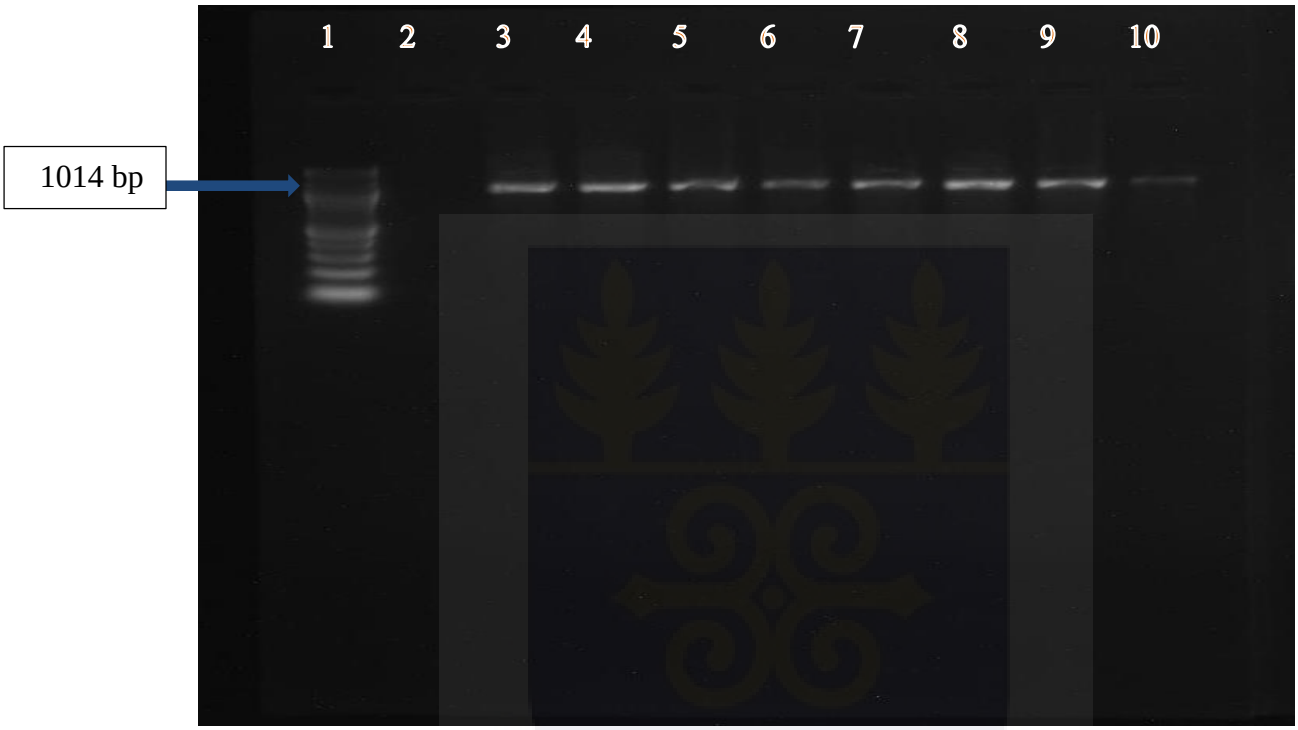

Figure 4.2: A representation of 2% Ethidium bromide-stained Agarose gel electrophoresis of some Precore/Core gene PCR amplicons. Lane 1 – 1 kbp molecular ladder; Lane 2- Negative Control (NC); Lane 3- Positive Control (PC) and Lanes 4-9 shows successfully amplified bands (1014bp).

4.4 HBV PRE S1/ PRE S2 GENE AMPLIFICATION (S2)

Amplification of this region was achieved in 15 samples out of the 26 samples used and 5 of these PCR positive samples were sent for sequencing.

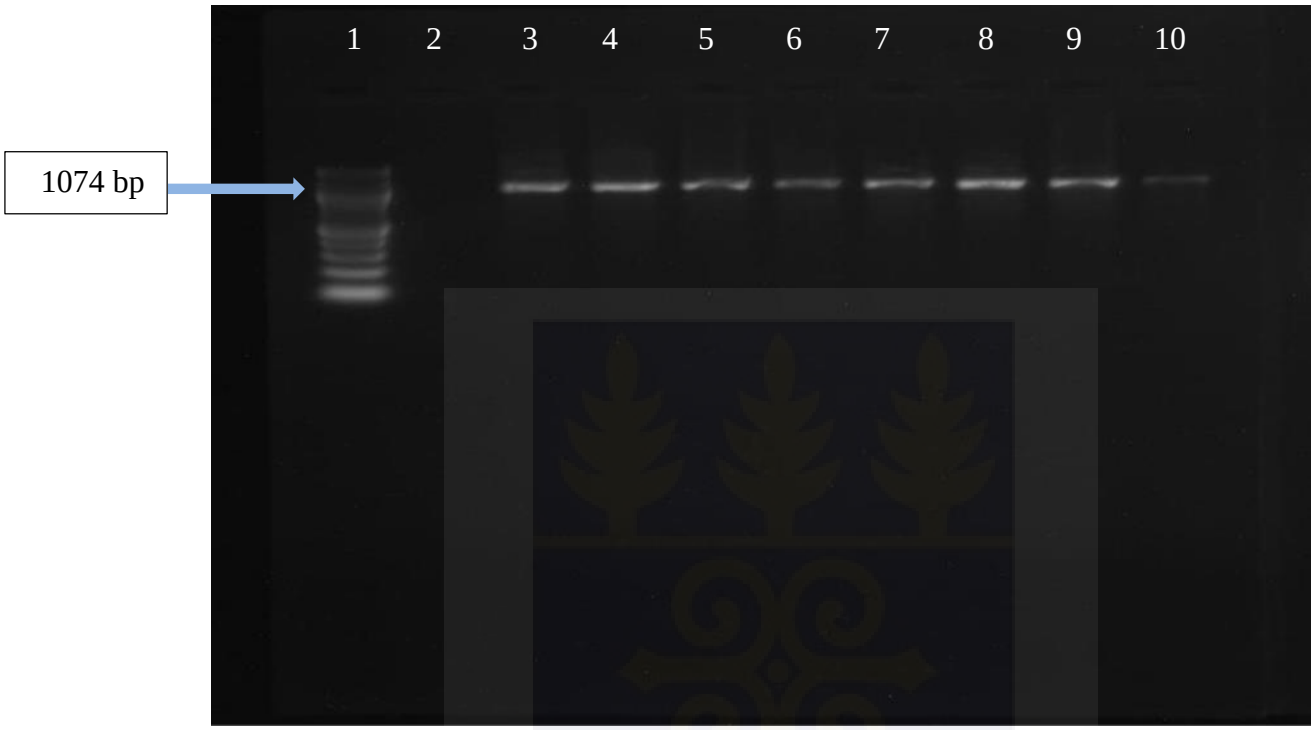

Figure 4.3: 2% Ethidium bromide-stained Agarose gel electrophoresis of some Precore/Core gene PCR amplicons. Lane 1 – 1 kbp molecular ladder; Lane 2- Negative Control (NC); Lane 3- Positive Control (PC) and Lanes 4-9 shows successfully amplified bands (1074bp).

4.5 NUCLEOTIDE SEQUENCE RESULTS FOR S GENE

4.5.1 GENOTYPE DETERMINATION

The S gene of 17 samples out of 26 samples were successfully sequenced and their genotypes were determined using the Stanford database and the jumping Profile Hidden Markov Model online genotyping tools. All sequences belonged to genotype E using the Stanford database whereas one sample (14S) belonged to genotype A/E whiles the others belong to genotype E using the jumping Profile Hidden Markov Model online genotyping tools (Table 4.3)

Table 4.3: Genotype distribution of successfully amplified samples.

STANFORD DATABASE			**jPHMM-HBV**	
ID	GEN0TYPE	SIM%	**ID**	GENOTYPE
2I	E	98.4	**2I**	E
3S	E	99.4	**3S**	E
6S	E	99.6	**6S**	E
8S	E	98.6	**8S**	E
10S	E	98.5	**10S**	E
9S	E	98.5	**9S**	E
9I	E	99.4	**9I**	E
12S	E	99.2	**12S**	E
13I	E	97.0	**13I**	E
13S	E	97.9	**13S**	E
14I	E	98.8	**14I**	E
14S	E	94.5	**14S**	A/E
15S	E	94.3	**15S**	E
16S	E	99.0	**16S**	E
18S	E	97.7	**18S**	E
2SG	E	98.9	**2SG**	E
6SG	E	99.5	**6SG**	E

A: Stanford Database (https://hivdb.stanford.edu/HBV/HBVseq/development/HBVseq.html)

B: Jumping Profile Hidden Markov Model(jPHMM –HBV)

(https://jphmm.gobics.de/submission)

4.6 SEQUENCE IDENTITY MATRIX AND PHYLOGENETIC TREE ANALYSIS

Sequence Identity Matrix (SIM) was generated for all the samples using the various genomic region (S gene (Table 4.4), Precore/Core gene (Table 4.5) and the PreS1/PreS2 (Table 4.6) gene. A Sequence Identity Matrix value of 1 or close to one shows that the sequences of those inmates are closely related. The colour codes used in the SIM plot shows the range of the values. Phylogenetic trees were constructed for all the genomic regions using the Neighbour joining method with Molecular Evolutionary Genetics Analysis tool (Mega 7) software with 1000 bootstrap replicate.

4.6.1 S GENE SIMILARITY INDEX MATRIX (S1)

Most of the samples had SIM values ranging from 0.940 -0.980 showing sequence relatedness. Sample 18S, 2SG, and 6SG were the most divergent sequences (Table 4.4). Two different phylogenetic trees were drawn. Four samples were repeated for one of the trees (2I and 6S and 9S and 12S) and this was purposely drawn to test the robustness of using the s gene for phylogenetic analysis (figure 4.4b). Most of these sequences were strongly clustered together (figure 4.4a and 4.4b).

Table 4.4: S Gene Sequence Identity Matrix

Seq->	2I	3S	6S	8S	10S	9S	9I	12S	13I	13S	14I	14S	15S	16S	18S	2SG_	6SG
2I	ID																
3S	0.956	ID															
6S	0.948	0.978	ID														
8S	0.958	0.98	0.97	ID													
10S	0.954	0.966	0.956	0.96	ID												
9S	0.935	0.927	0.916	0.929	0.929	ID											
9I	0.947	0.976	0.968	0.98	0.95	0.92	ID										
12S	0.944	0.941	0.929	0.941	0.944	0.956	0.933	ID									
13I	0.939	0.966	0.968	0.96	0.946	0.908	0.958	0.923	ID								
13S	0.93	0.928	0.924	0.932	0.934	0.913	0.923	0.931	0.912	ID							
14I	0.954	0.978	0.962	0.976	0.96	0.925	0.97	0.941	0.952	0.932	ID						
14S	0.919	0.922	0.913	0.918	0.92	0.891	0.911	0.899	0.905	0.886	0.922	ID					
15S	0.909	0.914	0.907	0.918	0.906	0.901	0.904	0.909	0.893	0.896	0.91	0.877	ID				
16S	0.952	0.956	0.946	0.96	0.954	0.929	0.951	0.945	0.938	0.93	0.954	0.907	0.914	ID			
18S	0.866	0.863	0.854	0.871	0.875	0.866	0.861	0.874	0.848	0.867	0.865	0.833	0.853	0.867	ID		
2SG	0.758	0.753	0.75	0.753	0.857	0.747	0.75	0.752	0.751	0.743	0.751	0.767	0.727	0.755	0.688	ID	
6SG	0.897	0.889	0.882	0.891	0.889	0.884	0.883	0.888	0.874	0.873	0.889	0.859	0.865	0.897	0.816	0.74	ID

Colour Code	Range
	0.978-0.980
	0.960-0.976
	0.950-0.958
	0.940-0.949
	0.922-0.937
	0.910-0.918
	0.901-0.909
	≤ 0.899

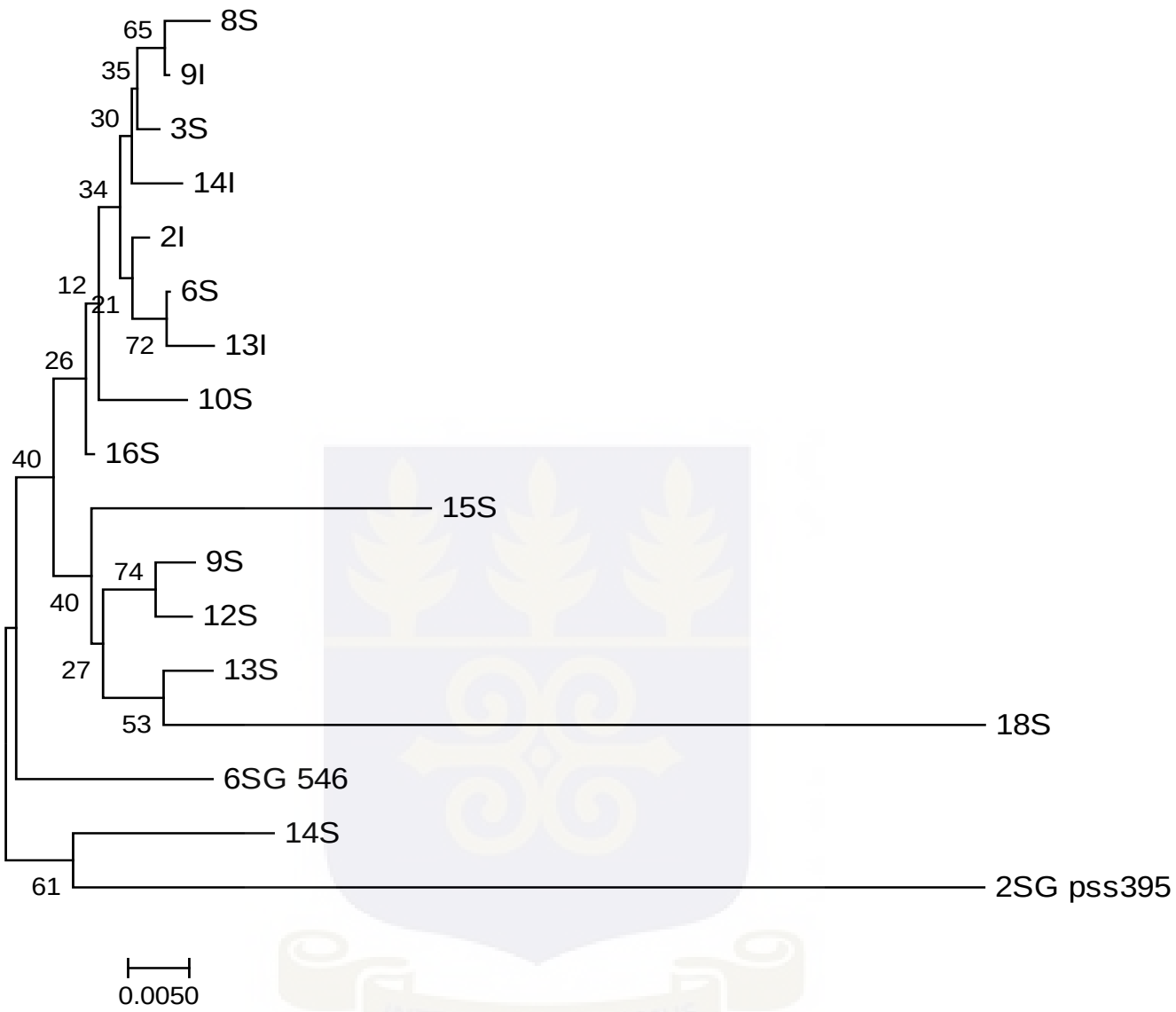

Figure 4.4a: Phylogenetic tree of the S gene from the central prison inmates using Neighbour joining method. Numbers represent sequence identity and the suffix "I" indicates inmates that were anti- HBcIgM positive.

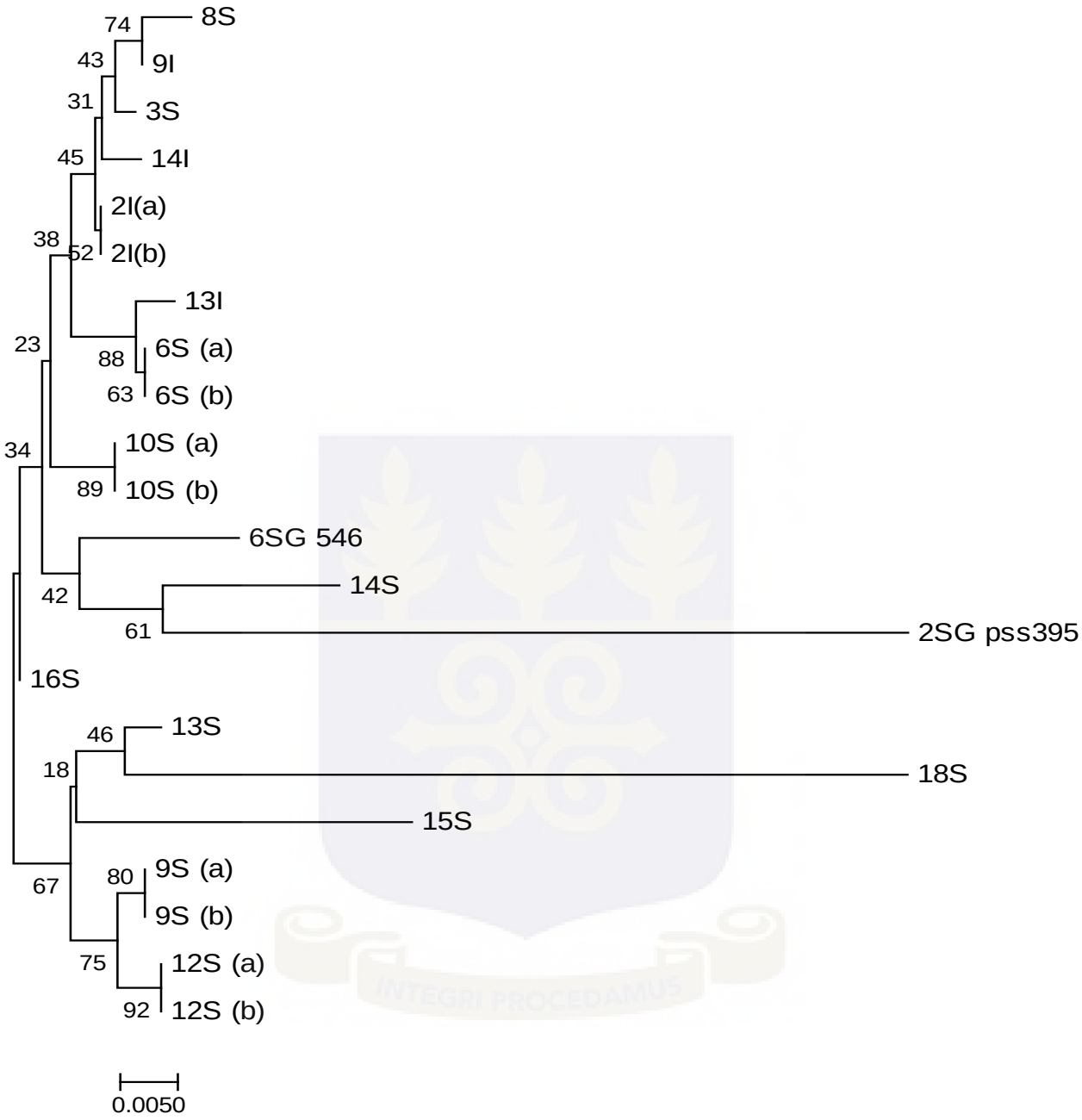

Figure 4.4b: Phylogenetic tree of the S gene from the central prison inmates using Neighbour joining method. Numbers represent sequence identity and the suffix "I" indicates inmates that were anti- HBcIgM positive. Samples 2I, 6S, 9S and 12S were repeated to test the robustness of using the S gene for phylogenetic analysis.

4.6.2 PRECORE/ CORE REGION SIMILARITY INDEX MATRIX (S1)

Eight samples were successfully sequenced out of which seven was used for analysis. One of the samples didn't give a good sequence so was excluded from the analysis. Sequences of samples 9I and 2I were closely related with a Similarity Index Matrix value of 0.971. Sample 14I's nucleotide sequence was also closely related to sequences of sample 2I and 9I (Table 4.5). The phylogenetic tree further supported the SIM values obtained with samples 9I and 21,14I and 2I and samples 2I and 9I (figure 4.5).

Table 4.5: Similarity Index Matrix showing Precore/Core Gene nucleotide sequence relatedness

Seq->	9I (9)	2I (8)	14I(4)	13I(5)	6S (1)	3S (2)	8S (3)
9I (9)	ID						
2I (8)	0.971	ID					
14I (4)	0.933	0.92	ID				
13I (5)	0.67	0.668	0.643	ID			
6S (1)	0.519	0.519	0.514	0.45	ID		
3S (2)	0.582	0.582	0.582	0.467	0.722	ID	
8S (3)	0.827	0.821	0.816	0.602	0.531	0.631	ID

Colour Code	Range
	0.930- 0.971
	0.933-0.92
	0.827-0.821
	0.722-0.602
	0.582
	0.531-0.514
	0.45-0.467

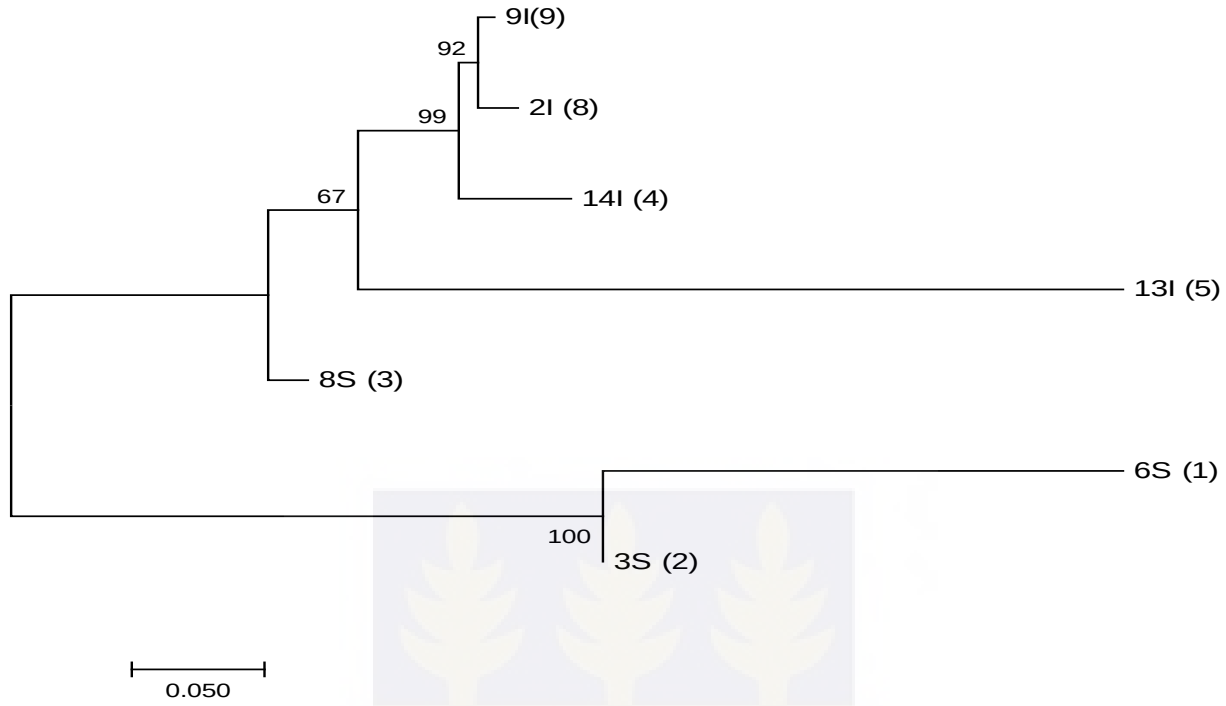

Figure 4.5: Phylogenetic tree of the Precore/ Core gene (S1) from the central prison inmates using Neighbour joining method. Numbers in parenthesis were the new identities for the samples from the previous study. Numbers represent sequence identity and the suffix "I" indicates inmates that were anti- HBcIgM positive.

4.6.3 PRE S1/ PRE S2 GENE SIMILARITY INDEX MATRIX (S2)

Sequences of this region were obtained for five samples. Sample 6S and 3S were closely related. Sequences of samples 8S and 3S, 8S and 6S were also related (table 4.6). The phylogenetic tree further supported the SIM values obtained (figure 4.6).

Table 4.6: Similarity Index Matrix showing Pre S1/ Pre S2 (S2) Gene nucleotide sequence relatedness.

Seq->	6S (1)	3S (2)	8S (3)	21 (8)	9I (9)
6S (1)	ID				
3S (2)	0.943	ID			
8S (3)	0.921	0.912	ID		
21 (8)	0.872	0.862	0.853	ID	
9I (9)	0.82	0.825	0.811	0.773	ID

Colour Code	Range
	0.943-0.912
	0.872-0.853
	0.825-0.773

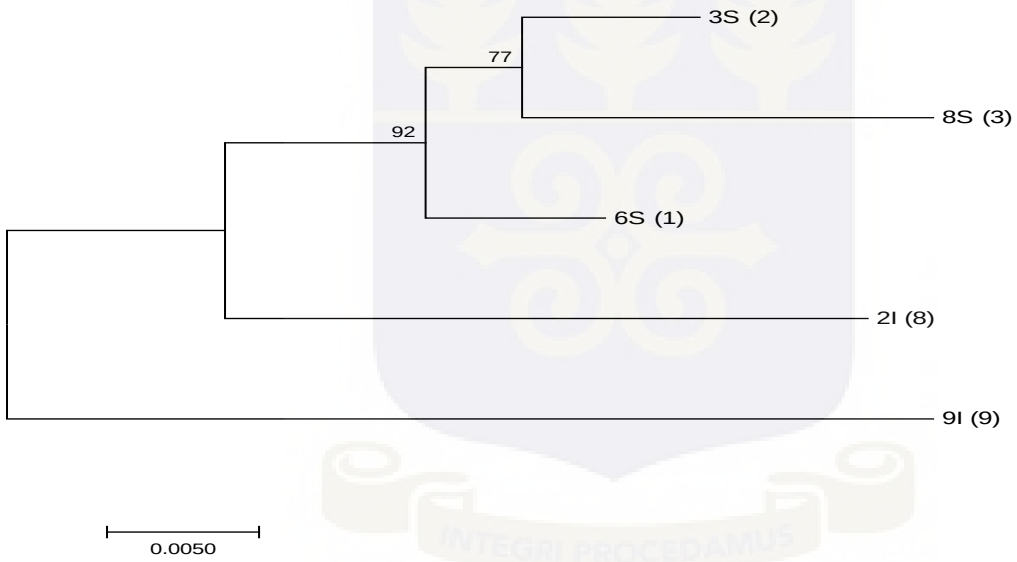

Figure 4.6: Phylogenetic tree of the PreS1/PreS2 (S2) from central prison inmates using the Neighbour joining method. Numbers in parenthesis were the new identities for the samples from the previous study. Numbers represent sequence identity and the suffix "I" indicates inmates that were anti- HBcIgM positive.

4.7 MUTATIONAL ANALYSIS

4.7.1 S GENE MUTATIONS

Mutational analysis was done using the Stanford Database and the jumping Profile Hidden Markov Model mutational analysis tool. Various mutations were seen in the small envelope protein of HBV (SHB) and some samples which showed some form of relatedness using the SIM values and the phylogenetic trees had some mutations in common. The L209V mutation was seen in all the amino acid sequences (table 4.7). The colour codes stand for different mutations that were common in other samples. For example the Q54KQ was seen in samples 2I and 10S.

Table 4.7: Table showing the different mutations in the SHB Mutations

SHB MUTATIONS	
2I (8)	L209V, P214T, G50*RW, Q51FLPS, Q54KQ, I110IL, I213Y
3S (2)	L209V, P214T, P211LP, I213*FIKLNY,
6S (1)	L209V, P214T, P211FI, I213*FIKLNY, L215EG, F212FL,
8S (3)	L209V, P214T, N52[n.d.], I213FINY, P203R,
9S	L209V, R73LR, P105AP, S113AS, P203PR,
9I (9)	L209V, P214T, L215E, I213FINY, L216K
10S	L209V P214T, P211LP, Q54KQ, T63IT, , I213NY,
12S	L209V, P214T, R73LR, I213Y
13I (5)	L209V, T63IT, S140LS, W156GW, G159EG, M197IM, I213*FLY, P214*FIKLNY, L215PR
13S	L209V, P214T, S55FV, P56R, S58[n.d.], R73DGHR, D144DE, T189IT, V190AV, I213Y
14I (4)	L209V, P214T, T57I, I68IN, T123AT, I213NY,
14S	L209V, T57[n.d.], I68IT, P70AP, I92IT, S114T, R122K, L127LP, T131N, S140T, S143T, S204NS, I213IK
15S	L209V, N59D, H60DN, P62T, S64ST, G71D, C76G, R78LR, R79HR, I82V, I86IS, L87LQ, I92IN, Q101HQ, P203R, I213IK
16S	L209V, P214T, I213FY,
18S	L209V, F80FS, M133L,
5S (2SG)	T63IT, P70AP, I92IT, V106GV, S114ST, R122KQR, L127P, T131N, S140*PQS,S143T, G159AGPR, W165GW, A166AG, S167FL, A168GS, R169P, L173LV, S174HQR, V177MR, P178A, V180GV, Q181*PQS, W182GW, F183FV, A184AGPR, G185GR, L186LV, S187FS, T189PT, V190G, W191RW, L192LV, S193FLS, V194GISV, M197K, M198K, W199G, Y200*E, P203AP, S204K, L205FL, Y206DV, N207H, L209V, S210KN, P211PS, I213Y, P214SY, L215E
24S (6SG)	Q54K, S55*PRS, P62[n.d.], R78QR, R79CR, P105PT, S114ST, S117RS, F134FV, C139*C, S140ST, C149*, A168AT, W172*W, L173I, V177GV, W182GW, L192FL, W199GW, Y200NY, Y206NY, L209V, F212FL

Because the S gene is completely overlapped by the Reverse Transcriptase gene (RT), some of the mutations seen in this genomic region were the RT mutation.

Table 4.8: Table showing the RT Mutations in the S gene

ID	**RT MUTATIONS**
2I (8)	W58LS, P59AITV, K60*K, A62AE, N118NT, L217R, Y221L, T222Y, S223R
3S (2)	L217R, L220F, Y221*FLY, T222NY, S223R
6S (1)	L217R, S219HL, L220F, Y221*FIKLNY, T222NY, S223R
8S (3)	K60[n.d.], L217R, L220FL, Y221*FLY, T222Y, S223R
9S	A113AG, N118HN, I121IS, C136CS, L217R
9I (9)	L217R, L220FL, Y221*FLY, T222Y, S223R, V224K, T225S
10S	P130Q, L217R, L220F, Y221*L, T222Y, S223R
12S	Y111NY, L217R, Y221L,T222Y, S223R
13I (5)	M164MR, D206DN, L217R, L220F, Y221FL, T222*FIKLNY, S223PT
13S	V63GV, Q67[n.d.], N71DN, S81*SW, L217R, Y221L, T222Y, S223R
14I (4)	N76KN, Y126HY, N131NS, L217R, L220FL, Y221*L, T222Y, S223R
14S	N65[n.d.], S78CS, L91IL, N118DN, I122NY, Y126HY, T128AT, L129LM, P130Q, N131DN, D134DN, N139Q, F148Y, F151Y, K154KQ, L217R, L220F, Y221*Y
15S	Q67R, S68*, T70Y, L72HL, W79*, V84G, H90R, H94HQ, H100HQ, R110RW, L217R, Y221*Y
16S	L217R, L220FL, Y221FL, T222Y, S223R
18S	P130Q, Y141F, L217R
5S (2SG)	S78CS, L80IL, L82LM, L91IL, V103IV, N118DN, I122HILN, Y126HY, T128AT, L129LM, P130PQ, D134DN, N139KQ, F148FLS, F151Y, M164LM, R167PR, K168EK, P170S, V173MR, S176KQR, F178FV, A181AG, Q182S, F183FV, S185KN, A186G, I187L, C188CW, S189FLS, V190GMRV, V191GV, R192CPRSW, R193PRT, A194AG, H197HP, C198W, L199LS, A200AG, S202HKNQRS, M204LM, D205E, D206E, V207G, V208GV, L209M, A211AG, S213AITV, V214*C, Q215P, L217R, S219AFSV, Y221L, T222FL, S223R
24S (6SG)	V63AV, P64T, T70[n.d.], N71D, L80LV, A87AV, F88FL, L91I, A113AD, I122IN, Y126DH, V142GV, F148FINY, G152GR, Y158N, S176NS, A181DN, S185RS, V190GV, A200AV, Y203NY, V207GV, V208EV, V214EV, L217R, Y221NY

4.7.2 PRECORE MUTATIONS

Three mutations were common in samples 3S and 8S and samples 2I and 9I also had three mutations in common. Sample 6S and 13I had no mutations in their sequence (table 4.9). No mutation was seen in the sequences of samples

Table 4.9: Table showing various Precore Mutations seen in the sequences

ID	Mutations
6S (1)	
3S (2)	L22LM, C23CR, G25AG, L27FL, W28CFLR, G29S
8S (3)	K21M, L22LM, C23CR, L24FL, G25AG, L27FL, W28*FHLQY, G29GV
14I (4)	C23CG, L27FL, W28*
13I (5)	
2I (8)	S13AG, C14H, P15G, T16I, V17D, Q18P, A19Y, S20K, K21E, L22F, C23G, L24A, G25T, W26AV, L27E, W28L, G29L
9I (9)	I9GR, C12*, T13A, C14L, P15F, V17S, Q18P, A19Y, S20C, K21T, L22Q, C23A, L24RS, G25H, W26S, W28L

4.7.3 CORE PROMOTER MUTATIONS

This was the most variable region with different unique sequences. Samples 2I, 9I, 3S, 8S, and 14I had one common mutation V13AV. Other common mutations were seen in some of the samples (table 4.10).

Table 4.10: Table showing various Core Mutations

ID	CORE MUTATIONS
2I (8)	V13AV, R28KR, E40D, E64D, W71GW, V72GV, N74V, D78DN, V85EV, N87GS, M93V, I97F, W102GW, I105FI, R112KR, L116I, E117EK, Y118NY, L119LW, V120GV, S121PS, F122FL, V124GV, P130PT, R133KR, T146N, R150KR, R152KR, , R181P, E182A, C185CG
9I (9)	K7HKNQ, A11AV, V13AV, L15ALSV, V27AV, D32DH, E40D, Q57HQ, E64DE, V72GV, N74V, N87S, I97F, L116I, E117EK, V124GV, T146N, D153., R154., R181P, E182A, S183AS
3S(2)	K7SY, F9GW, G10E, A11AV, V13GV, L15W, S17A, F18FI, P20D, D22V, F24Y, S26D, V27EK, L31Y, D32L, T33S, A34V, S35GW, A36AV, Y38I, R39S
8S (3)	K7*KNSTY, F9CFLW, V13GV, L15*, , E40D, A41G, A54AV, R56LMRW, A58AV, N74V, A80E, D83E, V85AS, N87RS, 142PT, T146N, R150RT, D153., R154., R160RT, R169IR, Q171HQ, R176K, Q179H, R181P, E182A, S183V, C185M
14I (4)	I3M, D4G, P5W, K7G, E8GR, F9G, A11AD, V13GV, L15LV, F18FV, V27AV, D32DN, Y38FY, E40D, L42LW, C48CG, I59ILM, C61CW, E64D, M66MV, N74V, A80AE, N87S, M93GV, I97F, R127HR, P130T, R133KR, T146N, D153., R154., R167IR, R169IR, R181P, E182A
13I (5)	K7KT, F9FL, L15*L, E40LQ, A41D, E43G, S44Y, E46., H47., C48., S49., P50., H51., H52., T53., A54., L55., R56., Q57., A58., I59?

CHAPTER FIVE

5.0 DISCUSSION

5.1 NUCLEOTIDE SEQUENCE ANALYSIS

In all 17 samples were successfully sequenced for the S gene, 8 samples were sequenced for the Precore/Core gene (S1) and PreS1/Pre S2 sequences were also obtained for 5 samples. With the exception of sample 14S which was determined to be a recombinant strain belonging to genotype A/E by using the jumping Profile Hidden Morkov Model online tool , all other sequences belonged to genotype E which is the predominant genotype present in the Ghanaian population. The observation of genotype E in this study is in accordance with studies done by Geretti *et al*., (2010); Garmiri *et al*., (2009); Zahn *et al*., (2008) in Ghana and other African countries; Ayiku, (2015) who also reported the predominant genotype to be genotype E. Reports also indicate that some HBV infected individuals in Ghana also harbor the A/E genotype and it is therefore not surprising that one of the inmates (14S) was infected with the recombinant strain (Garmiri *et al*., 2009; Candotti, Danso, & Allain, 2007; Thien *et al*., 2006). The fear is that this inmate who is HBeAg positive and highly infectious is likely to transmit this recombinant HBV strain to other inmates within the prison. This inmate reported to have been forced to have sex with another inmate within the prison and this may serve as a means of further spread of the virus among inmates who have sex with him. Twelve inmates out of the 17 successfully sequenced samples for the S gene admitted to have been forced into this sexual act and most of this inmates were HBeAg positive. Indeed there appears to be a great deal of sexual activity within inmates even though this phenomenon is discouraged by prison authorities (Adjei *et al*., 2006).

5.2 S GENE NUCLEOTIDE SEQUENCE ANALYSIS

The S gene results from the similarity index matrix clearly showed that most of the sequences were identical and these were also clustered together on the phylogenetic tree. This can be explained by how well conserved the S gene is (Datta *et al*., 2007; Thien *et al*., 2006, Gous, 2006). Due to the highly conserved nature of the S gene, sequences of samples from different population may be found to be closely related to each other thereby impairing the ability to distinguish transmission dynamics ((Datta *et al*., 2007). For instance from the S gene Similarity Index Matrix Plot (Table 9), the sequence of sample 9S was closely related to 12S with a SIM value of 0.956. Further investigation using demographic data revealed that sample 9S is positive for HBeAg marker, has been incarcerated for 1 year in this prison and has had a record of only shared blades with other inmates within the prison. Inmate with the identity 12S on the other hand had spent just 1 month in this prison, was HBeAg negative but HBsAg positive and never had a record of being expose to any risk behavior whiles in prison. However this inmate had one sexual partner before he was incarcerated and he admitted that he never used a condom during sex. Clearly this infections were from different epidemiological background yet their sequences were found to be closely related. Similar observation was made with samples 8S and 9I which are from two different prisons and never had a record of ever being in the same prison. In the case of samples 3S and 14I which are all from the same prison, a SIM value of 0.98 is indicative of possible transmission from sample 3S to sample 14I. From the demographic data, sample 3S was HBeAg positive, had spent 5 months in this prison and had a history of sharing blades with other inmates. Sample 14I was positive for both HBeAg and anti-HBc IgM, had spent 3 years in this prison, and had no record of being involved in any risk behavior while in prison. This observation is quite astonishing because anti-HBc IgM denotes recent infection. It is possible

that this inmate held back information from the interviewers for many reasons some of which may be due to fear that their information will be shared with others (Apa *et al*., 2012). Samples 6S, 10S and 13I also had closely related sequences suggesting possible transmission from either sample 6S or sample 10S to sample 13I. Sample 6S is HBeAg positive, had spent almost 6 months in prison, and had shared blade with other inmates, he also used the same tattoo piercing implement that other inmates used to tattoo himself while in prison. He also had a blood covenant with another inmate.

This inmate was obviously at a higher risk of getting infected and also served as a good source of HBV transmission within this prison. Sample 13I is positive for both HBeAg and anti-HBc IgM, spent almost 7 months in this prison and reported that he has been forced to have sex with other inmates. Sample 10S was not reactive to both HBeAg and anti-HBc IgM, had spent barely 5 months in this prison and has also reported to have been forced to have sex with other inmates. It is still surprising that one inmate had reported that he had the tattoo while in prison and used an implement that others used for the same purpose yet he was the only person who reported this. Others may also be involved in this behavior but held back that information from the interviewer. Similar observations which suggest possible intra prison transmission was seen for samples 2I and 9I who after examination of demographic data revealed that this two inmates were at a point in time in the same prison until sample 2I was transferred to another prison. Data was however not obtained on the duration of stay of this inmates in the same prison as well us the time 2I was transferred. Sequences of samples 6S and 3S were also closely related

The PreS1/PreS2 region for 5 samples were sequenced; this includes samples 2I (8), 3S (2), 6S (1), 8S (3) and 9I (9). From the SIM plot and the phylogenetic tree, sequences of sample 6S and sample 3S were closely related with a SIM value of 0.943. Same observation was made for

samples 8S and sample 3S. This suggest possible transmission of HBV between this three inmates who have all in one way or the other involved in at least one risk behavior.

Although the S gene has been established to be highly conserved, different specific variability levels for the S gene (genotype, subgenotype, and subtype), in conjunction with mutations, can provide enough confidence to prove transmission events (Datta *et al*., 2007).

5.3 PRECORE/ CORE GENE NUCLEOTIDE SEQUENCE ANALYSIS

Out of the 8 samples whose precore/core gene was sequenced, one sample was not part of the samples analyzed because it was not in the best of shape for analysis. The sequences of sample 2I and 9I were closely identical with a SIM value of 0.971 and this observation was supported by the phylogenetic tree for the PreS1/PreS2 gene. A careful scrutiny of the demographic revealed that this two inmates were at a point in time in the same prison until sample 2I was transferred to another prison. Data was however not obtained on the duration of stay of this inmates in the same prison as well us the time 2I was transferred. An interesting observation was seen with how sequences of sample 141 was closely related to sequences of samples 2I and 9I. Although sample 2I and 9I had history of being in one prison at a point in during their incarceration, there was neither a history of being in the same prison with samples 2I and 9I nor previous incarceration for sample 14I. This relatedness was also seen in the S gene of these inmates and must be further investigated.

5.4 MUTATIONAL ANALYSIS AND POSSIBILITY OF HORIZONTAL TRANSMISSION OF MUTANTS

5.4.1 S GENE RT MUTATIONS

Viral resistance mutations have numerous distinguishing characteristics, including its association with virologic breakthrough during the drug therapy; its response in patients exposed to the HBV drugs, its capacity to confer resistance to the virus in vitro and lastly its reversion to wild-type sequence in the absence of the selective antiviral pressure due to reduced replication capacity of the mutant compared to the wild-type virus (Liu et al., 2014). HBV. Mutations in the MHR also influence the antigenicity and can impair virion secretion consequently leading to HBsAg detection failure (Oluyinka *et al*., 2015). In this study, mutational analysis of the overlapping S and polymerase genes of HBV of all the I7 successfully amplified samples was done. The L217R, occurs outside the domain of the ‘‘a” determinant and can therefore presume this L217R mutation may perhaps be linked with detection failure this occult hepatitis B status in some individual (Oluyinka *et al.*, 2015). Bottecchia *et al*., (2008) on the other hand, reported that this mutation could be responsible for the low response to adefovir. Another common mutation that runs through all the samples with the exception of samples 9S, 13I, 14S, 15S and 18S, was the S223R. 8 of the samples also had a common T22Y mutation. The presence of common mutations in samples whose sequences were closely related (3S & 14I, 6S, 10S & 13I, 3S & 6S and 3S and 8S) further confirms transmission dynamics within these inmates. Sequences of samples 8S and 9I were closely related and also had 4 mutations in common.

5.4.2 S GENE SHB MUTATIONS

All the samples had the L209V mutation and this mutation has been reported to be an exclusive characteristics of mostly HBV genoyptpe E sequences that are available in the GenBank (Mathet *et al*., 2006). The role of L209V is still under investigation however it is speculated that antibodies directed to the vaccine-derived HBs antigen (subtype adw) might not be effective at the neutralization of some HBV L209V S-mutated genotype E isolates (Mathet *et al*., 2006). Ten of the inmate samples also had a common mutation (P214T). Even though the mutations were not evenly distributed across the samples, samples whose sequences were closely related still had common mutations that suggest horizontal transmission of those mutant strains. Mutations in the MHR can affect anti-HBsAb binding and result in discrepant reactivity between diagnostic assays thereby tumbling or invalidating the sensitivity of detection. A T131N mutation was found in the "a" determinant of the S gene in sample 14S and was also reported by Olinger, (2008) and Geretti *et al*., 2010). This same sample had a silent mutation at amino acid position 142 (S143T). These mutations were also reported by Olinger, (2008) and may interfere with the HBsAg detection when using commercial HBsAg kits. Sample 14I had a T123A mutation has been shown to escape detection using the Architect HBsAg assay (Geretti *et al*., 2010).

5.4.2 PRECORE MUTATIONS

Even though whole genome is the gold standard for establishing events of transmission, analysis of nonoverlapping, fast-evolving regions is usually recommended in when whole genome sequences are unavailable. About 67% of the HBV genome is overlaps, leaving distal X/preC/partial core regions non overlapped. These sections of the genome encrypt essential RNA structural elements needed for HBV replication. Thus, one can assume that high variability

in these regions might have negative selection pressure (Datta *et al*., 2007). Precore stop codon W28* mutation at position 1896 was observed in two of the samples investigated (8S &14I). This is in agreement with a previous study by Oluyinka *et al*., (2015) and Zahn *et al*., (2008) in Nigeria and Ghana respectively. This mutation induces a stop codon generating a non-functional HBeAg and adversely affect HBeAg production hence the determination of HBeAg status. Again closely related sequences shared common mutations.

5.4.3 CORE MUTATIONS

In this study, this region was the most variable region with many substitution. All the sequences of the samples investigated had unique mutations at various positions. However closely related sequences shared some common mutations.

5.5 LIMITATION OF THE STUDY

Poor amplification of the PreS1/PreS2 region as well as the precore/core region for most of the samples hampered analysis. This could have given a stronger and richer picture of the level of sequences relatedness among inmates and also serve as a concrete basis arguing of on-going transmission within the prisons especially to clear the doubt of inmates who claimed they had not been involved in any risk behavior during their over 6 months stay in the prison yet were anti-HBc IgM positive. Assuming the PreS1/PreS2 gene sequence for sample 14I was good, it could have further helped resolve the dilemma of this sample being closely related to samples 2I and 9I (at different prisons currently but were once in the same prison that is different from where sample 14I currently stays).

5.6 CONCLUSION

The predominant genotype in this study was genotype E and one inmate harboured a recombinant strain belonging to the A/E genotype. Similarity Index Matrix and phylogenetic analysis of the S gene, PreS1/PreS2 and precore/core genes demonstrated ongoing transmission of HBV within prison and HBeAg antigenemia positive inmates were mostly responsible for recent infections in other inmates. In some instances anti-HBc IgM inmates transmitted HBV infection to other anti-HBc IgM inmates as in the case of samples 2I and 9I. Common mutations were observed in samples whose sequences were closely related suggesting horizontal transmission of HBV mutants within prison inmates. Of note is the L209V and L217R SHB and RT regions respectively which runs across the sequences. W28* codon mutation was also present in two inmates (8S & 14I). From the demographic data there is a great deal of risk behaviours such as blade sharing, piercing, tattooing and more especially sodomization. This is a bad omen to the community at large as this inmates will be eventually released into the community and continue HBV transmission. Transmission dynamics investigations using molecular analysis may be impaired by the continuous transfer of inmates from one prison resulting in completely different sequences obtained from inmates within the same prison.

5.7 RECOMMENDATION

This intra prison transmission of HBV infection demonstrated in this study argues for expansion of prevention programs to reduce HBV transmission in prisons. Inmates should constantly be educated on the risk behaviours associated with HBV transmission and the ways that this transmission can be prevented. It is also recommended that prisoners are screened for HBV upon

admission, and this should be followed with periodic, regular screenings. Cases should be notified where required and such cases be put on antiviral therapy if necessary.

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APPENDICES

APPENDIX I: PRISON INMATE QUESTIONNAIRE

National Health and HIV Survey of Prison Inmates and Prison Officers in Ghana

PRISON INMATES' QUESTIONNAIRE

SCHOOL OF PUBLIC HEALTH

CONSENT FORM

We, the Ghana AIDS Commission (GAC), Ghana Prisons Service, National AIDS/STI Control Programme (NACP) and German International Cooperation (GIZ) [Regional Coordination Unit for HIV & TB], working in partnership with the School of Public Health, University of Ghana are undertaking a national survey of Prison Inmates and Prison Officers to determine the health status of prison inmates. The main aim of this study is to assess the prevalence and situation of HIV, HBV and other key illnesses such as malaria, diabetes, hypertension and underweight amongst inmates.

The study comprises behavioural and biological components. The behavioural component involves one-on-one interview to assess inmates' knowledge, attitude and behaviours on HIV and STIs, and general health situation, while officers will also be interviewed to explore how they perceive inmates' attitude and

behaviours regarding HIV and general health situation. The biological part will involve drawing anonymous blood sample from inmates. From the blood sample, rapid tests will be done at the prisons to determine the presence HIV, HBV, Malaria, and Diabetes. Afterwards, plasma for confirmed HIV or HBV infections will be sent to the laboratory for the determination of recent infections and the transmissibility of HBV. Additionally, your blood pressure (BP) will be checked for hypertension, and weight & height measurements taken to estimate Body Mass Index. The study will use very experienced counsellors and biomedical personnel who have handled many similar cases before, and so if you test positive to HIV or HBV, you will be counselled and referred for care and treatment and follow up supportive counselling.
This research will give you the chance of knowing, free of charge, your health situation in terms of HIV and Hepatitis B infections, Diabetes, Blood pressure and BMI (whether you are over or under-weight). This will give you the knowledge to enable you to improve your health and get medical support. Additionally, the results of this study will enable the Ghana AIDS Commission and its partners to know the prevalence of HIV and STIs and the general health status of inmates in order to institute appropriate measures to secure the health of prison inmates in Ghana.

We are therefore asking you to participate in this study to contribute to the national effort in preventing HIV and improving the health of inmates through the information you will provide.

We assure you that any information you provide will be strictly confidential; and will be used only for the purposes of this research and never be used against you.

Your participation is voluntary, and you may stop the interview at any time.

Do I have your permission to continue? [___] Yes [___] No
Respondent's signature: _____

[If Yes] I, _____ (interviewer) certify that the respondent has given permission to participate in the study.
Interviewer's signature: _____

If you have any questions concerning this study, please feel free to contact the Principal Investigator:

Dr. Angela El-Adas, MD, MPH
Director-General, Ghana AIDS Commission
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PRISON INMATES' QUESTIONNAIRE

A

Questionnaire Number : [___|___|___|___]

Name of Prison : _____

Type of Prison : 1 Maximum security (Ankaful New)
2 Medium security (Nsawam)
3 Central Prison
4 Local Prison
5 Agricultural Camp

Region : _____

Date of Interview : Day:_____ Month :_____ Year:_____

Time of Interview : Started _____ Finished _____

Name of Interviewer : _____

Interview Status : 1. FULLY Completed 2 PARTLY Completed 3 Refused

Returned for FBS Test : 1 Yes 2 No

Comments on Interview :
_____

Interview Tests

Participant asked & given Verbal Consent to : 1. Questionnaire : 1 Yes 2 No 2. HIV Test : 1 Yes 2 No 3. HPV Test : 1 Yes 2 No 4. Malaria Test : 1 Yes 2 No 5. Diabetes Blood Sugar Test : 1 Yes 2 No 6. Blood Pressure Test : 1 Yes 2 No 7. Weight & Height Measure : 1 Yes 2 No Signature of Interviewer: _____	**Test Results:** **HIV**= 1 POS. 2 NEG. 3 INDE. **HBV**= 1 POS. 2 NEG. **MALARIA** = 1 POS. 2 NEG. 3 INDE. **BLOOD PRESSURE** = _____ **BLOOD SUGAR (RBS)= _____** Blood Sugar (FBS) Test Required? 1 Yes 2 No If Yes, Blood Sugar (FBS) = _____ **HEIGHT (m)** = _____ **WEIGHT(kg)** = _____ **BMI** = _____ kg/m^2

INSTRUCTION: Please circle the appropriate answer the respondent gives for all closed-ended questions, and write legibly in the spaces provided answers to all open-ended questions.

NO.	QUESTIONS	CODING	SKIP TO
1.0	**SECTION 1 SOCIO-DEMOGRAPHIC**	**"I am going to start by asking some questions about your background"**	
1.1	What sex is the interviewee? ***[Circle from observation]***	Male 1 Female 2	
1.2	How old are you? ***[Age at last birthday]***	Age in years [___|___] Don't Know 88 No response 99	
1.3	In which country have you lived for most of your adult life?	Ghana 1 Nigeria 2 United Kingdom (UK) 3 Other..... 4 No Response 99	→ 1.5 → 1.5 → 1.5

1.4	In which region did you live for most of your adult life before coming to prison?	Greater Accra 1 Ashanti 2 Western 3 Central 4 Brong Ahafo 5 Northern 6 Upper West 7 Upper East 8 Volta 9 Eastern 10 No response 99	
1.5	What is your nationality?	Ghanaian 1 Non Ghanaian (Foreigner) 2 No response 99	
1.6	Have you ever attended school?	Yes 1 No 2 No Response 99	→ **1.9**
1.7	Up to which level of schooling did you attend?	Primary 1 Junior High 2 Senior High 3 College / University 4 No Response 99	
1.8	What was the highest class you completed?	..... No response 99	
1.9	What is your religion?	Christian 1 Traditionalist 2 Muslim 3 No Religion 4 Other..... 5 No Response 99	
1.10	How often do you attend a religious meeting?	More than once a Week 1 Once a Week 2 Few times a Month 3 Few times a Year 4 No response 99	
2.0	**SECTION 2** **PRISON ENVIRONMENT**	**"I would like to ask a few questions about your time in prison"**	

2.1	Are you on remand or convicted?	On Remand /awaiting trial 1 Convicted 2 No Response 99	→ **2.3**
2.2	How long is your prison sentence?	Number of Years [___|___] Number of Months [___|___] Less than one month 66 No response 99	
2.3	How long have you been in prison on this sentence or remand /awaiting trial?	Number of Years [___|___] Number of Months [___|___] Less than one month 66 No response 99	
2.4	How long have you been in <u>this</u> prison?	Number of Years [___|___] Number of Months [___|___] No response 99	
2.5	How many other prisons have you been in for this sentence?	Number of Prisons [___|___] Less than one month 66 No response 99	→ **2.7 if ans=0**
2.6	Can you give the names of other prisons you have been in during this sentence & for how many months / years	.....(__ yrs __ mths) <1 mth 66 .....(__ yrs __ mths) <1 mth 66 .....(__ yrs __ mths) <1 mth 66 .....(__ yrs __ mths) <1 mth 66 No response 99	
2.7	Have you been in prison before this sentence?	Yes 1 No 2 No response 99	→ **3.0**
2.8	How many times have you been in prison before?	Number of Times Before [___|___] No response 99	
3.0	**<u>SECTION</u> 3** **HEALTH IN PRISON**	**"Next I will ask you some questions about the general health situation in this prison"**	SKIP TO

3.1	What are the common illnesses in this prison? ***Do not read out answers.*** ***Up to 3 multiple responses possible.*** ***Probe by asking: Any others?***	Malaria 1 Fever 2 Headache 3 Stomach Aches 4 Pneumonia 5 Diarrhoea 6 Dysentery 7 Piles 8 Urethral Discharge 9 HIV/AIDS 10 Jaundice 11 Tuberculosis (TB) 12 High Blood Pressure 13 Skin Diseases 14 Diabetes 15 Other ……..16 Other ……..16 Other ……..16 Don't Know 88 No Response 99	
3.2	What illnesses have you suffered from in this prison? ***Do not read out answers.*** ***Up to 3 multiple responses possible.*** ***Probe by asking: Any others?***	Malaria 1 Fever 2 Headache 3 Stomach Aches 4 Pneumonia 5 Diarrhoea 6 Dysentery 7 Piles 8 Urethral Discharge 9 HIV/AIDS 10 Jaundice 11 Tuberculosis (TB) 12 High Blood Pressure 13 Skin Diseases 14 Diabetes 15 Other ……..16 Other ……..16 Other ……..16 Don't Know 88 No Response 99	
3.3	Have you been coughing frequently (every hour) for the last 2 weeks?	Yes 1 No 2 Don't know 88 No response 99	
3.4	Have you been having fever and chills frequently in the last 2 weeks?	Yes 1 No 2 Don't know 88 No response 99	→ **3.6**
3.5	Have you had regular headaches with the fever?	Yes 1 No 2 Don't know 88 No response 99	

3.6	Have you been sweating a lot at night for the last 2 weeks?		Yes 1 No 2 Don't know 88 No response 99	
3.7	Have you experienced loss of appetite in the last 2 weeks?		Yes 1 No 2 Don't know 88 No response 99	
3.8	Have you been waking up to urinate more than 2 times a night in the last week?		Yes 1 No 2 Don't know 88 No response 99	
3.9	Have you noticed yourself losing a lot of weight in the last month?		Yes 1 No 2 Don't know 88 No response 99	
3.10	Have you ever had Sexually Transmitted Infections?	OUTSIDE PRISON Yes 1 No 2 No Response 99	WITHIN PRISON Yes 1 No 2 No Response 99	
3.11	Have you experienced any pains or ulcers in the genital areas?	OUTSIDE PRISON Yes 1 No 2 No Response 99	WITHIN PRISON Yes 1 No 2 No Response 99	
3.12	Have you experienced pain during urination?	OUTSIDE PRISON Yes 1 No 2 No Response 99	WITHIN PRISON Yes 1 No 2 No Response 99	
3.13	Have you ever experienced an unusual discharge?	OUTSIDE PRISON Yes 1 No 2 No Response 99	WITHIN PRISON Yes 1 No 2 No Response 99	
3.14	How did you treat it?	Hospital treatment 1 Herbal treatment 2 Not treated 3 Self-medication 4 Other ..... 5 Don't know 88 No Response 99	Hospital treatment 1 herbal treatment 2 Not treated 3 Self-medication 4 Other..... 5 Don't know 88 No Response 99	
3.15	Does the prison offer health services		Yes 1 No 2 Don't know 88 No response 99	

4.0	**SECTION 4 : HIV RISK - BLOOD CONTACT**	**"I would like to ask you some questions about your experiences in prison"**		
4.1	Is there access to blades / razors for shaving in this prison?	Yes 1 No 2 Don't know 88 No response 99		
4.2	Do you share blade / razor *(for shaving or hair cut)* with other inmates in this prison?	Yes 1 No 2 No response 99		
4.3	Have you ever been tattooed or pierced?	OUTSIDE PRISON Yes 1 No 2 No Response 99	WITHIN PRISON Yes 1 No 2 No response 99	→ **4.5**
4.4	Was the same implement used for other people?	OUTSIDE PRISON Yes 1 No 2 No Response 99	WITHIN PRISON Yes 1 No 2 No response 99	
4.5	Have you ever made a (direct) blood covenant?	OUTSIDE PRISON Yes 1 No 2 No Response 99	WITHIN PRISON Yes 1 No 2 No response 99	
4.6	Do you know other inmates in this prison who have made a blood covenant?	Yes 1 No 2 No Response 99		
5.0	**SECTION 5 HIV RISK – INJECTING DRUG USE**			
5.1	Do you know inmates in this prison who use drugs?	Yes 1 No 2 Don't know 88 No response 99		→ **5.7**
5.2	How often do they use drugs?	Every week 1 Every month 2 Few times a year 3 Once every year 3 Less than once every year 4 Don't know 88 No response 99		

5.3	Which drugs do you often see used? ***Read out each answer.*** ***Tick number if 'yes'*** ***Multiple responses allowed*** ***Also Specify Combinations***	('Wee') Marijuana 1 Crack / Cocaine 2 Heroin 3 Alcohol 4 Pethidine 5 Valium 6 Other..... 7 Don't Know 88 No response 99		
5.4	Do you know people in this prison who inject drugs?	Yes 1 No 2 Don't know 88 No response 99		→ **5.7**
5.5	Do these people have to share needles?	Yes 1 No 2 Don't know 88 No response 99		
5.6	Which drugs are usually injected by inmates? ***Read out each answer.*** ***Tick number if 'yes'*** ***Multiple responses allowed***	Heroin 1 Crack / Cocaine 2 Amphetamine 3 Pethidine 4 Other..... 5 Don't Know 88 No response 99		
5.7	Have you ever used drugs?	<u>OUTSIDE PRISON</u> Yes 1 No 2 No Response 99	<u>WITHIN PRISON</u> Yes 1 No 2 No Response 99	→ **6.0**
5.8	How often have you used drugs?	<u>OUTSIDE PRISON</u> Every week 1 Every month 2 Few times a year 3 Once every year 3 Less than once every year 4 Don't know 88 No response 99	<u>WITHIN PRISON</u> Every week 1 Every month 2 Few times a year 3 Once every year 3 Less than once every year 4 Don't know 88 No response 99	

5.9	Which drugs have you used? ***Read out each answer.*** ***Tick number if 'yes'*** ***Multiple responses allowed***	OUTSIDE PRISON ('Wee') Marijuana 1 Heroin 2 Crack / Cocaine 3 Alcohol 4 Pethidine 5 Valium 6 Other..... 7 Don't Know 88 No response 99	WITHIN PRISON ('Wee') Marijuana 1 Heroin 2 Crack / Cocaine 3 Alcohol 4 Pethidine 5 Valium 6 Other..... 7 Don't Know 88 No response 99	
5.10	Have you ever injected drugs?	OUTSIDE PRISON Yes 1 No 2 Don't know 88 No response 99	WITHIN PRISON Yes 1 No 2 Don't know 88 No response 99	→ **6.0**
5.11	How often have you injected the drugs?	OUTSIDE PRISON Every week 1 Every month 2 Few times a year 3 Less than every year 4 Don't know 88 No response 99	WITHIN PRISON Every week 1 Every month 2 Few times a year 3 Less than every year 4 Don't know 88 No response 99	
5.12	Which drugs have you injected? ***Read out each answer.*** ***Tick number if 'yes'*** ***Multiple responses allowed***	OUTSIDE PRISON Heroin 1 Crack / Cocaine 2 Amphetamine 3 Pethidine 4 Other..... 5 Don't Know 88 No response 99	WITHIN PRISON Heroin 1 Crack / Cocaine 2 Amphetamine 3 Pethidine 4 Other..... 5 Don't Know 88 No response 99	
5.13	Have you ever had to share needles with other drug users?	OUTSIDE PRISON Yes 1 No 2 No Response 99	WITHIN PRISON Yes 1 No 2 No response 99	
6.0	**SECTION 6** **HIV RISK – SEXUAL CONTACT**	**"Some of the following questions will be about your personal life and sexual behaviour."**		

6.1	What is your marital status?	Single (never married) 1 Married – One Partner (monogamous) 2 Living together 3 Married – 2+ Partners (polygamous) 4 Divorced 5 Separated 6 Widowed 7 No Response 99	
6.2	Before you came to prison – did you ever have other casual sex partners?	Yes 1 No 2 Don't know 88 No Response 99	→ **6.5**
6.2a	If Yes, in total, with how many different people have you had casual sex with during the last 12 months before you came to prison	Number casual partners [___|___] No response 99	
6.3	How often did you have casual sex partners – in the 12 months before you came to prison?	More than once a Week 1 Once a Week 2 Few times a Month 3 Few times a Year 4 No response 99	
6.4	How often was a condom used with a casual partner?	Never 1 A few times 2 Most times 3 Every time 4 Don't know 88 No Response 99	
6.5	Before you came to prison, did you ever have sex with a commercial sex worker?	Yes 1 No 2 Don't know 88 No Response 99	→ **6.8**
6.6	How often did you have casual partners – in the 12 months before you came to prison?	More than once a Week 1 Once a Week 2 Few times a Month 3 Few times a Year 4 No response 99	

6.7	How often was a condom used?	Never 1 A few times 2 Most times 3 Every time 4 Don't know 88 No Response 99	
6.8	At this prison – Have you had sex with other inmates?	Yes 1 No 2 No Response 99	→ **6.17**
6.9	Have you had oral sex with other inmates – in this prison?	Yes 1 No 2 No Response 99	→ **6.11**
6.10	How many times have you had oral sex in this prison in the last three months?	..... times 1 Don't Know 88 No response 99	
6.11	Have you had anal sex with other inmates – in this prison?	Yes 1 No 2 No Response 99	→ **6.13**
6.12	How many times have you had anal sex in this prison in the last three months?	..... times 1 Don't Know 88 No response 99	
6.13	How often was a condom used?	Never 1 A few times 2 Most times 3 Every time 4 Don't know 88 No Response 99	
6.14	Have you ever paid/received money or goods (e.g. cigarettes, drugs, blades) for sex in prison?	Yes 1 No 2 Don't know 88 No Response 99	
6.15	Have you ever engaged in sex with other inmates in this prison who inject drugs?	Yes 1 No 2 Don't know 88 No Response 99	

6.16	Did you use condom during your last sex in this prison?	Yes 1 No 2 Non-Applicable 77 No Response 99	
6.17	Have you heard of inmates being forced to have penetrative sex?	Yes 1 No 2 Don't know 88 No response 99	→ **6.19**
6.18	How often do you think inmates are forced to have penetrative sex within this prison?	every few days 1 every few weeks 2 every few months 3 Once a year 4 Don't know 88 No response 99	
6.19	Have you ever been forced to have anal or oral sex?	**Anal Sex** Yes 1 No 2 No response 99 **Oral Sex** Yes 1 No 2 No response 99	→ **6.21**
6.20	How many times have you been forced to have penetrative sex in prison?	..... Times No response 99	
6.21	Have you ever forced anyone to have anal or oral sex?	**Anal Sex** Yes 1 No 2 No response 99 **Oral Sex** Yes 1 No 2 No response 99	→ **7.0**
6.22	How many times have you forced anyone to have penetrative sex in prison?	..... Times No response 99	
6.23	How would you rate your chance of getting infected with HIV in this prison?	High risk 1 Medium risk 2 Low risk 3 No risk 4	
7.0	**SECTION 7: HIV/AIDS KNOWLEDGE AND ATTITUDE**	**I am going to ask you some questions about HIV/AIDS. Please do not worry about getting the right answer, just say what you think is true**	

7.1	Have you ever heard of an illness called AIDS?	Yes 1 No 2 No Response 99	
7.2	Can people reduce their chance of getting the HIV by having just one uninfected sex partner who has no other sex partners?	Yes 1 No 2 Don't know 88 No Response 99	
7.3	Can people get HIV from mosquito bites?	Yes 1 No 2 Don't know 88 No Response 99	
7.4	Can people reduce their chance of getting HIV by using a condom every time they have sex?	Yes 1 No 2 Don't know 88 No Response 99	
7.5	Can people get HIV by sharing food with a person who has HIV/AIDS?	Yes 1 No 2 Don't know 88 No Response 99	
7.6	Can people reduce their chances of getting HIV by not having sexual intercourse at all?	Yes 1 No 2 Don't know 88 No Response 99	
7.7	Can people get HIV because of witchcraft or other supernatural means?	Yes 1 No 2 Don't know 88 No Response 99	
7.8	Do you think that HIV can be transmitted by injection with used needle?	Yes 1 No 2 Don't know 88 No Response 99	

7.9	Do you think that HIV can be transmitted by common use of razor blades?	Yes 1 No 2 Don't know 88 No Response 99	
7.10	Do you think that HIV can be transmitted by common use of tooth brushes?	Yes 1 No 2 Don't know 88 No Response 99	
7.11	Do you think that HIV can be transmitted by tattooing?	Yes 1 No 2 Don't know 88 No Response 99	
7.12	Do you think that HIV can be transmitted by sharing blood in brotherhood/ sisterhood rituals?	Yes 1 No 2 Don't know 88 No Response 99	
7.13	Is it possible for a healthy looking person to have HIV?	Yes 1 No 2 Don't know 88 No Response 99	
7.14	If a member of your family became sick with AIDS, would you be willing to care for her or him in your own household?	Yes 1 No 2 Don't know 88 No Response 99	
7.15	If you knew that someone is HIV infected, would you eat with him or her?	Yes 1 No 2 Don't know 88 No Response 99	
7.16	If you knew that someone is HIV infected, would you continue to meet or associate with him/her?	Yes 1 No 2 Don't know 88 No Response 99	

7.17	If you knew that someone is HIV infected, would you share a cell with him/ her?	Yes 1 No 2 Don't know 88 No Response 99	
	Have you ever had an HIV test, before this survey?	Yes 1 No 2 Don't know 88 No Response 99	→ **7.21**
7.19	If yes when was the last time you were tested?	Less than 12 months ago 1 1 to 2 years ago 2 2 or more years ago 3 Don't know 88 No Response 99	
7.20	What was your Test Result? - ***You do not have to share this if you are not comfortable***	Positive 1 Negative 2 Don't know 88 No Response 99	
7.21	Do you want to know your HIV status at the end of this Survey?	Yes 1 No 2 No Response 99	
7.22	If you were HIV positive do you think that you would feel comfortable disclosing your status in the prison?	Yes 1 No 2 No Response 99	
7.23	Are HIV/AIDS services offered in this prison?	Yes 1 No 2 Don't know 88	→ **7.25**
7.24	What activities or services have you seen? ***Answer yes/no for each service***	(a) Education - Yes 1 No 2 (b) HIV Testing - Yes 1 No 2 (c) Treatment - Yes 1 No 2 (d) Support Groups - Yes 1 No 2 No Response 99	
7.24 a	Are these services accessible to every prisoner?	Yes 1 No 2 Don't know 88	

7.25	Do you need information about HIV/AIDS in the prison?	Yes 1 No 2 Don't know 88 No Response 99	
7.25 a	Do you need information about other health issues in the prison?	Yes 1 No 2 Don't know 88 No Response 99	
7.26	Do you need information about other health issues in the prison?	Yes 1 No 2 Don't know 88 No Response 99	
7.27	Do think condoms should be made accessible in prisons?	Yes 1 No 2 Don't know 88 No Response 99	
7.28	What would you recommend to help reduce the risk of HIV in this prison? ***Multiple responses possible.***	Better Access to Condoms 1 Access to clean needles 2 Access to Razor Blades so not share 3 Educational Sessions 4 Educational Leaflets 5 Prevent Forced Sex from Happening 6 Other..... 7 Don't know 88 No response 99	
Comments on Interview:			

Time Finished _____
Thank you for your help in answering this questionnaire.

APPENDIX II: SPECIMEN BANKING FORM

AGREEMENT TO PARTICIPATE IN SPECIMEN BANKING

Study Title:
National Health and HIV Survey of Prison Inmates and Prison Officers in Ghana

You have agreed to participate in a study known as **National Health and HIV Survey of Prison Inmates and Prison Officers in Ghana**, sponsored by the Ghana AIDS Commission and partners.

You are being asked to sign this second consent form to indicate if you are willing to allow the samples collected solely for research purposes, which will be referred to as your 'Specimen', to be saved or 'banked' for use in future research studies. At this time, we do not know what future research studies may be done using your Specimen. The specimen bank will be maintained by the Clinical Virology Laboratory, University of Ghana Medical School.

Your signature below will allow your Specimen to be stored in the specimen bank, with the possibility that it will be used in future research studies. It is very unlikely that any future research performed using your Specimen would benefit you directly, but it may provide important medical knowledge that could be helpful in understanding the transmission of viral pathogens in prisons. Information about how your Specimen will be used and protected is as follows.

A. Confidentiality and Privacy of Medical Record
If you sign this form, you give the Clinical Virology Laboratory permission to store your Specimen in a controlled specimen bank, along with portions of your personal health information collected related to the Main Study. This information could be used to link the specimen back to you. Clinical Virology Laboratory will protect your confidentiality by making sure that no information that could be used to identify you will be used or disclosed by Clinical Virology Laboratory without your authorization or without legally required protections in place. The following rules will apply:

The Clinical Virology Laboratory in charge of the specimen bank will determine for which research studies to release all or part of your Specimen.

1) In most cases, your Specimen will be 'de-identified'; that is, the researcher who is given your
Specimen will not be given enough information to identify you. In these cases, you will not be contacted prior to your Specimen being released to the researcher.

2) Your authorization would not be required for researchers to use partially de-identified (in accordance with legal standards)
Specimens for future research studies; however, such researchers would be required to sign a Data Use Agreement, which would protect your privacy by limiting how they could use your Specimen.

3) If an approved researcher wants to use your Specimen and feels that is important that he or she be able to identify you to a)
Collect information about you that was not collected as part of your participation in the Main Study; or, b) Collect additional samples of your blood then this would be considered using an identifiable Specimen (meaning the Specimen could be linked back to you). In that case, someone associated with Clinical Virology Laboratory will contact you to provide further information about the proposed study so you can decide whether you will agree to participate. If you decide to participate, you will then be contacted by a researcher for the new study and asked to sign a separate consent form for that study. Clinical Virology
Laboratory will apply national legal standards in making decisions about who can review your records in preparing for the study and about who can contact you to provide information about the new study.

4) It is possible that your Specimen could be used for future research purposes without your consent or authorization if a committee of people who know about research, privacy and medical ethics (such as the Noguchi Memorial Institute for Medical Research Institutional
Review Board or the Ghana Health Service Ethical Review Committee) decided that use of your information is necessary and that use of it would be of low risk to you and your privacy.
Clinical Virology Laboratory will ensure that all specimens stored at the Clinical Virology Laboratory will be kept confidential and only shared by the Clinical Virology Laboratory in accordance with the above rules; no other people, including relatives or
Ghana Prisons Officials will have access to the stored samples or information about them without your written consent. Appropriate physical and computer security measures will be maintained to limit access to Specimens.
Papers or articles written by Clinical Virology Laboratory researchers which are based on studies involving your Specimen will not identify you by name.

B. Control and Ownership of the Specimen(s)
By consenting to participate in the specimen bank, you authorize the banking of your Specimen for research conducted in accordance with the rules described above. If you should have a need for the Specimen at some later date for a medical purpose, it usually can be removed from the specimen bank for that purpose unless there is no specimen left.

C. Withdrawal of Your Consent
If you decide at some time in the future that you no longer wish your stored Specimen to be used in future studies, you have the right to request that the Specimen be withdrawn from the specimen bank. However, withdrawal cannot be guaranteed and may be impossible. For example, it is possible that the Specimen might no longer be identifiable as belonging to you, or that it might already have been released for research studies and used up. To request withdrawal of the Specimen from the specimen bank, please write to Dr. Kwamena Sagoe, Clinical Virology Laboratory, Department of Microbiology, University of Ghana Medical
School, P. O. Box 4236, Accra (Mobile: 0277408528).

D. Length of Storage
Specimens in the bank will be stored for an indefinite period of time, until research funding is exhausted or the Specimen is no longer usable.

E. Signature
You have the right to refuse to sign this form. Refusing to sign this form will not affect your participation in the Main Study or your access to health services in the prison. If you chose to sign, your signature below indicates that you have read this form and discussed it with researchers associated with the Main Study, and that you wish to participate in the specimen bank in accordance with the terms described in this form.

---------- …….
Participant's Signature Date

……. ……..
Witness (only if consent presented orally) Date

…… ……
Participant's legal representative Date
(If patient unable to sign)

APPENDIX III: AMINO-ACID-TABLE-SINGLET-CODE