Review Article
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Epidemic risk of *nisseria meningitidis* capsular switching between sero-groups: the effect of immunological pressure

Abstract

Meningococcal meningitis is a serious infection and is associated with considerable mortality and morbidity in various parts of the world. The disease has a global epidemiology but sub-Saharan Africa bears the highest burden of the disease. The bacterium known to switch its capsular polysaccharide from one sero-group to the other in response to immunological pressure after mass vaccination campaign through genetic material transformation. Antigenic variation of lipopolysaccharides may arise from phase variation of one or more enzymes involved in the synthesis of the oligosaccharide chain. On the other hand, the bacterium can involve allelic exchange of genes or gene fragments from imported DNA. Thereafter, sero-converted strain can cause epidemic of meningococcal meningitis with newly introduced sero-groups which warn policy makers and professionals which helps for further decision making.

Keywords: capsular switching, immunological pressure, meningococcal meningitis, polysaccharide capsule, sero-group

Volume 2 Issue 5 - 2015

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Received: October 27, 2015 | **Published:** November 16, 2015

Introduction

The meningococcal disease is caused by bacteria *Neisseria meningitidis* and it is a leading cause of septicemia and meningitis. There are substantial cyclical fluctuations in meningococcal disease incidence; occurrence; and epidemics. The disease causes an acute inflammation of the protective membranes covering the brain and spinal cord. The bacterium is commensal and can be recovered from nasopharyngeal swab human samples Findlay & Redfield.[1]

The polysaccharide capsule is a responsible for meningococcal meningitis virulence. Based on the capsular polysaccharide genetic structural differences; there are 13 N. meningitidis sero-groups have been identified and the isolates responsible for human disease associated with sero-groups A; B; C; W135; and Y Joseph et al.;[2]

2004; Swartley et al.,[3] Sero-groups B and C are responsible for infections in Europe and America; and sero-groups A and C infections common in Africa and Asia Beddek et al. [4]. Capsular polysaccharides are the outermost antigens on the meningococcal surface and the prime target for mucosal and humoral immunity. From these important thirteen sero-groups; common strains are encapsulated with sero-groups A; B; C; W135 and Y and cause more than 90% of the invasive disease worldwide. Non-sero-group able meningococcal meningitis are nonpathogenic; but capsule production in bacterial meningococcal meningitis strains can switch on and off at a high frequency causing capsular switching Swartley et al.,[5] and can overcome immune detection of the host. Evidence indicate that the loss of a capsular polysaccharide enhances the ability of meningococci to colonize nasopharynx and help to avoid human defense systems Findlay & Redfield.[1] indicating importance of capsule to be detected by host's immune system during spread of infection. Meningococcal meningitis use mechanisms such as slipped-strand mis-pairing and acquisition of an insertion sequence in genes responsible for synthesis or transport of the polysaccharides involved in loss of capsule expression.

Epidemiology

The only natural reservoir of *N. meningitidis* is the human nasopharyngeal mucosa and transferred from one person to another by close contact or via respiratory droplets at a distance up to one meter Nelson et al.,[6] Meningococcal disease occurs word-wide as endemic infection. Globally; bacterial meningitis affects approximately 1.2 million people each year and causes almost 170;000 deaths per year WHO.[7] The distribution of the sex difference reveals a slight predominance of the disease among male patients and the disease exhibit seasonality with large number of cases during winter. From the public health important sero-groups; sero-groups B and C cause the majority of infections in developed countries; while sero-groups A and; to a small amount; C contribute to infection in developing countries Caugant.[8]

Sub-Saharan African countries have a special epidemiological pattern associated with the disease. This region; designated as "meningitis belt"; which extends a line of sub-Saharan countries stretching from Senegal to Ethiopia Pollard et al.,[9] In meningitis belt; meningococcal disease caused by sero-group A occurs in periodic recurrent waves. The disease attack rate rises at the end of the dry season and declines rapidly after the beginning of the rainy season Riedo et al.,[10] According to WHO Global Alert and Response (GAR); from January 1 to May 12 2013; 9; 249 suspected cases of meningitis were recorded. From this; 857 were death with a case fatality ratio of 9.3 percent have been reported from 18 of the 19 African countries under enhanced surveillance WHO.[7]

In Ethiopia; meningococcal meningitis was reported for the first time in 1901; followed by outbreaks reported in 1935 Slaterus et al.,[11] Epidemiological studies on the spread of these epidemics in Ethiopia suggest the introduction of meningococcal disease first in western part of Africa. The meningococcal epidemics were thought to have spread from West Africa to Ethiopia following the line of meningitis belt. The epidemic of 1989 that occurred in the eastern part of Africa is believed to be spread by pilgrims returning from Mecca during the religious trip. Since the introduction of meningococcal disease in Ethiopia; the disease has remained endemic with frequent outbreaks. The outbreaks prior to 2001 occurred mostly in the north western; western and south western parts of Ethiopia; the areas that are traditionally included in the meningitis belt. However; outbreaks in 2001 and afterwards have extended to the eastern parts of the country as well WHO.[7]

Bacteriologic studies during the 1981-1983 epidemic period showed that serogroup A and C were dominating Moore[12] while; during 1988-1989 epidemic; serogroup A; with one isolate of B was the most prevalent serogroup Tekle et al.,[13] Serogroup A was the cause of the reported epidemic of meningitis in Ethiopia. In cases were recorded in 60 woredas (local municipalities) across 14 zones of South National and Nationality People Region (SNNPR) and Oromia; with increase of cases in 16 woredas of SNNPR and Oromia WHO [7]. Woredas with high cases of meningitis were Arbaminch Zuria; Halaba; Hawassa town; Dale; Shebedino; Gorche; and Wonsho in SNNPR; and Arsi Negele; Shalla; Shashemene Town; Shashemene Rural; Dodolla; Siraro; Wondo; and Gedeb Assassa in Oromia Region. During this outbreak; Ethiopia introduced ACW 135 polysaccharide meningococcal vaccines. There was no data on sero-conversion from known serogroup after mass vaccination campaign carried out previously in the country. Continuous surveillance system can be used to detect possible capsular switching after this vaccination to set further control mechanisms.

Immune response to *Neisseria meningitidis* infection

The first immunological defense against meningococcal infection associated with mucosal immunity and it's very effective in preventing clinical disease. For this; bacterium evolved several mechanisms to evade mucosal host immune response Virji.[14] The other is importance of complement proteins activation in response to meningococcal meningitis infection which is demonstrated by increased risk of meningococcal disease in individuals with deficient in complement proteins Jarvis & Griffis,[15] Antibody is potential activator of complement during defense against meningococcal disease.

During infection with meningococcal disease; adaptive immunity can be developed either by *infection encountered*with pathogenic *N. meningitidis* sero-group or infection with non-pathogenic one which can induce cross protective immunity. This opportunity resulted in use of naturally acquired immunity by immunization to prevent occurrence of disease. Vaccines are now available for some; but not all; meningococcal sero-groups. Plain polysaccharide vaccines can protect from disease caused due to sero-groups A; C; W135 and Y; and used for many years but cannot produce enough immune response in young children. This problem of immune response in children is averted by development of conjugate meningococcal vaccines which can offer good protection in the very young age. As vaccine development progresses and broader immunization with capsular polysaccharide conjugate vaccines becomes a reality; the ability to switch capsular types become important implications and currently the challenge in controlling and prevention of the disease Swartley et al.,[5]

Evolution of *Neisseria meningitidis* complex

The meningococcus possesses an outer membrane and is protected by a polysaccharide (Ps) capsule. The composition of this capsule is used to differentiate meningococci into sero-groups. The capsule of sero-groups B; C; W; X and Y contains sialic acid of different structures; while the sero-group A capsule contains α-1; 6-linked N-acetyl-D-mannosamine-1-phosphate Brehony et al.,[16] All capsules are immunogenic in humans; except the sero-group B capsule. The different sero-groups of meningococci vary in epidemiological features and immunogenicity. The disease caused by sero-group B is particularly got special attention due to its challenge concerning vaccine development. The outer coating capsule on sero-group B bacteria does not induce an immune response; because it closely resembles human cells which can help the bacterium to mimic human cells.

The development of universal vaccines against *N. meningitidis* has been a major challenge; mainly because of strain diversity and molecular mimicry Helena et al.,[17] In order to overcome immune detection; meningococci have evolved several mechanisms to change their surface components. Structural/antigenic variation of these molecules is one strategy and can involve allelic exchange of genes or gene fragments from imported neisserial DNA. This can occur frequently in *N. meningitidis* as it is naturally competent and readily takes up DNA from its environment Van der Woude & Baumler.[18] Another surface modulations can occur through phase variation; a process involving on and off expression of genes. Reversible insertion of mobile elements which involve tracts of repetitive DNA sequences that occur either upstream of a gene or within an open reading frame through a loss or gain of individual nucleotides. Antigenic variation of LPS on the other hand may arise from phase variation of one or more enzymes involved in the synthesis of the oligosaccharide chain; or by modification of LPS; by the addition of sialic acid Kahler & Stephens.[19]

N. meningitides may be capsulate or encapsulated when isolated from carriers; whereas blood and cerebrospinal fluid (CSF) isolates are invariably capsulated. The capsule is responsible for survival of the bacteria in the blood through rendering resistant to antibody complement-mediated killing process and hindering opsonic and non-opsonic phagocytosis Castilla et al.,[20] In the same way; certain LPS structures of the meningococci (L3; L7 and L9) can also contribute to immune evasion and are found more frequently in blood isolates when compared with carriage isolates. The carriage isolate tends to express L1; L8 and L10 LPS immunotypes Van der Woude & Baumler.[18] On the other hand; adhesion and invasion events bacterium can be affected by both capsule and certain immunotypes of LPS. The incorporation of sialic acids into the capsule and LPS enables bacteria to become less visible to the immune system as sialic acids are also commonly present on host cell surfaces.

The most striking mimicry; occurs in sero-group B capsule as α-linked sialic acid homopolymer is structurally identical with a component of human neural cell-adhesion molecule (NCAM); crucial for functional plasticity of the central and peripheral nervous systems Hill et al.,[21] Genetic similarities in the structures of the capsule loci of sero-groups B; C; W and Y (but not sero-group A) apparently favor horizontal exchange of portions of the capsule biosynthetic operon between different sero-groups resulting in capsule switching Swartely et al.,[5] As a consequence; any naturally acquired and vaccine-induced anti-capsular antibodies become ineffective in controlling or preventing the spread of the pathogen.

Risk of capsular switching on epidemics

Based on its characteristic; meningococcal epidemiology is affected by it is highly flowing and major fluctuations in the occurrence of endemic disease. Meningococcal sero-group distribution is highly regional and cyclical based on epidemiological distribution and previous intervention taken to control specific outbreak Harrison et al.,[22] Capsular switching is one of a genetic mechanism that allows meningococcal meningitis to change its capsular phenotype and as a result; outbreaks can begin or maintain by this method. This allows immunologic escape from immunity to the original sero-group and the escape sero-group becomes dominant causing epidemics. Capsular switching occurs when a person's nasopharynx is cocolonized with meningococcal strains and phase immunological pressure that forces the bacterium to change it is capsular genetic component to overcome immune detection Barroso et al.,[23]

Capsular switching appeared to be responsible for an outbreak of sero-group W-135 disease during the 2000 Hajj in Mecca; Saudi

Arabia and following this; the epidemic strain spread globally causing an outbreak in Burkina Faso Lancellotti et al.,[24] Capsular switching was also occured in the 1990s during an eruption of sero-group B disease in Oregon and sero-group C strains originate at genetically indistinguishable from the original sero-group B strain occurred during the outbreak.

One mechanism that infectious agents use to avoid host immune responses and cause infection is antigenic variation. Serotype replacement has been observed since the routine use of meningococcal conjugate vaccine began in the United States in 2000 Harrison.[25] A major concern is that; with mass vaccination using vaccines that do not include protection against all of the major meningococcal sero-groups; there could be an increase in the incidence of meningococcal disease caused by strains not included in the vaccine. This occurs through the mechanisms of capsular switching or capsular replacement from the previously known sero-groups to the newly introduced one.

Incidence of meningococcal disease can also increases in association with changes in non-capsular outer membrane proteins of sero-group B meningococci. The observation has great implications for sero-group B meningococcal outer-membrane protein–based vaccines in the prevention of disease. An increase in the incidence of sero-group C and Y meningococcal occurred in Maryland in association with an extensive antigenic shift in outer membrane proteins of the bacteria. Horizontal gene transfer led to major antigenic changes in FetA; and the porA gene in the case of sero-group C. But; in the case of sero-group Y; major antigenic changes caused by horizontal gene transfer which involve three outer membrane protein genes.

Capsular Switching

Antigenic diversity between *N. meningitidis* strains resulting from DNA transformation and result in subsequent capsular genes recombination. The genetic mechanisms involved in the switching of capsular genes from B to C followed by switching from C to W135 have been identified; but the recombination sites associated with these events have not been clearly defined Beddek et al.,[4] Almost all meningococcal disease is caused by bacterial organisms expressing one of the five important capsular antigens. Meningococci of sero-groups B; C; Y; and W-135 express capsules composed of polysialic acid or sialic acid linked to glucose or galactose and the capsule of group A *N. meningitidis* is composed of N-acetyl mannosamine-1-phosphate. These capsules are capable of protecting the bacterium against opsonophagocytosis during disseminated infection Zhu et al.,[26]

The different sialic acid (sero-groups B; C; Y; and W-135) and nonsialic acid (sero-group A) capsular polysaccharides expressed by *N. meningitidis* are major virulence factors and are used as epidemiologic markers and vaccine targets. In *N. meningitidis;* the horizontal transfer of *siaD* genes encoding polysialyl transferases has been shown to result in capsular sero-group switching in vitro Vassiliki & Nikos.[27] The capsule-synthesis gene cluster of meningococcal consist of different regions: genes that are required for polysaccharide synthesis comprise region A Edwards & Clarke,[28] Swartley *et al*.,[3] lipid modification genes associated with region B; region C; responsible for genes required for polysaccharide transport region D is involved in lipopolysaccharides synthesis region D′ is a condensed form of region D and region E is the functions of gene homologue present in region E which is unknown Peltola.[29] Region A contains genes specific with variants of the siaD gene required for synthesis of the sialic-acid-containing capsules of sero-group B; C; Y and W-135; and the myn genes essential for the expression of a sero-group A capsule Claus *et al*.,[30] Swartley et al.,[5]

Capsule switching in *N. meningitidis* is known to occur via horizontal DNA transfer between meningococcal meningitis strains. Due to this; antigenic variants can be introduced by allelic gene replacement of the siaDgenes and the variants can be selected by specific immunity against the original capsular antigen Lancellotti et al.,[24] Capsule switching events within the same clonal complex arise frequently with no alteration in virulence which enhances the system of surveillance by molecular typing of isolates; particularly after sero-group-specific vaccination.

Capsular switching from virulent sero-group C and Y lineages to sero-group B with clonal expansion is one mechanism by which replacement sero-group B disease could occur after the introduction of meningococcal conjugate vaccine Harrison et al.,[31] According to Beddek and his coworkers study; capsular switching has not occurred after the introduction of monovalent sero-group C conjugate vaccines; but the broader sero-group coverage of meningococcal conjugate vaccines could conceivably have a larger effect Beddek et al.,[4]

Sero-group C meningococci with genotypes similar to those of capsular sero-group B clonal strains causing epidemics have been found to occur as a result of capsule replacement. Recombinant strains showing sero-group B or C previously described to occur as a result of capsule-switching between sero-groups by genetic mechanism Harrison et al.,[22] Sero-group W-135 clone emerged in 2000 to cause outbreaks of meningococcal disease among Hajj pilgrims and subsequently caused large epidemics in parts of sub-Saharan Africa and many parts of the world via case collections Vassiliki & Nikos.[27] Genetically; the epidemic clone belonged to the sequence type (ST)-11 clonal complex which is typically associated with invasive sero-group C meningococcal strains. This suggests that capsular switching had occurred by gene transfer. This indicates that sero-groups not covered by meningococcal conjugate vaccine (MCV4) could emerge by a similar mechanism.

Capsular switching has been observed since licensure of the pediatric heptavalent pneumococcal polysaccharide protein conjugate vaccine (PCV7). Capsular switching of virulent lineages of *N. meningitidis* of sero-groups A; C; Y; and W-135 that are currently covered by MCV4 could lead to the selection of additional virulent sero-group B strains. Shift of antigenic structure with change of capsule polysaccharide is a main strategy for bacterial meningitis to evade a host immune detection. Accordingly; the molecular techniques confirm the possible capsule replacement in and between the important four sero-groups of meningococci (Ulrich *et al*.; 2000). In New Zealand; emergence of an invasive W: 2a:P1.7-2; 4 sequence type 11 (ST-11) an uncommon sero-group of of *N. meningitidis* strain was investigated for its genetic origins and molecular typing of 107 meningococcal isolates with similar serotyping characteristics determined for genetic relationships and W: 2a:P1.7-2; 4 strain emergency via capsule switching from a group C strain (C: 2a:P1.7-2; 4) Ulrich et al.,[32] Sequence analysis demonstrated that at least 45 kb of DNA was involved in the recombination; including the entire capsule gene cluster. The gene carried by the W: 2a:P1.7-2; 4 strain contained the introduction of new sequence element IS *1301*; from five variants of *oatWY* found in group W135 strains which belongs to the carriage-associated ST-22 clonal complex suggesting that the source of the capsule genes carried by the invasive W: 2a:P1.7-2; 4 strain is carriage associated Amanda et al.,[33]

Capsule switching reported in Canada and Spain after mass vaccination campaigns Alcala et al.,[34] and in the Czech Republic during an epidemic situation Kriz et al.,[35] An increasing number of meningococcal sero-group C strains were observed in Spain in the second half of the 1990s associated with C: 2b:P1.5; 2 ST8 strains

for the first until 1999 and continue with strain C: 2a:P1.5 ST11 isolates Berron et al.,[36] Barroso et al.,[37] The introduction of conjugate vaccine against sero-group C in 2000 resulted decline in the number of cases of sero-group C from 9 in 1998 to 0 in 2007 with quite stable sero-group B cases (not more than 6 to 11 annually) until 2005; and increasing to 16 in 2006 and 24 in 2007 Castilla et al. [20]. The emergence of B: 2a:P1.5 strains; coinciding with a progressive extension of meningococcal C vaccination; indicate strong immunological pressure may be selecting such types of strains which can evade the immune response produced during vaccination. Even though the frequency of the capsular replacement is not known; replacement of sero-group C 2a:P1·5; ST-11 by sero-group B 2a:P1·5; ST-11 meningococci may easily occur in population immunized with meningococci sero-group C strain.

In 2010; Burki Nafaso introduces the first meningococcal sero-group A conjugate vaccine and in 2012; Burki Nafaso reported increases in *N. meningitidis* sero-group W; which was as a result of sero-group replacement after introduction of sero-group A conjugate vaccine MacNeil et al.,[38] Therefore; the concern should be taken into account when introducing a monovalent vaccine is virulent isolates expressing the capsule targeted by the vaccine acquire genes coding capsule from its environment by horizontal gene transfer and which helps to evade the immune system.

In Brazil; prolonged epidemic of B:4 *N. meningitidis* infections was experienced during 1988–1999. In 1990 and 1994; mass vaccination was performed by using a vaccine consisting of a sero-group B outer membrane vesicle (B: 4:P1.15) and a sero-group C polysaccharide Terezinha et al.,[39] During 1993–1994; a total of 4 C: 4 *N.*

Meningitidis isolates were identified in samples from patients in Rio de Janeiro State. The presence of class 3-PorB strains from the isolates suggested the possibility of B to C capsular switching. Since 2000; the number of cases of sero-group C disease has steadily increased; reaching 90% of laboratory-confirmed cases in 2009 Barrow et al.,[40] The proportion of C:4 isolates increased from 2% (1988–1999) to 25% (2000–2009). Molecular characterization was used to confirm whether C:4 strains arose as a result of B:4 capsular switching Cohen et al.,[41] These all newly evolved *N. meningitidis* sero-groups which previously uncommon were an indication of possible sero-conversion between sero-groups by gene transfer which is risk for epidemic occurrence.

Acknowledgments

None.

Conflicts of interest

None.

Funding

None.

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