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MINIREVIEW

Significance of Meningococcal Hyperinvasive Clonal Complexes and their Influence on Vaccines Development

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Abstract

Neisseria meningitidis is a commensal of human nasopharynx and humans are the only known reservoir and host of this bacterium. It is also known as a dangerous and devastating pathogen, and infection with *N. meningitidis* may lead to rapidly progressing septicemia or meningitis. These severe infections, called invasive meningococcal disease (IMD), are one of the major public health threats worldwide. IMD may occur sporadically, but also in outbreaks, epidemics, and pandemics. Most of the IMD cases in the world are caused by isolates of genetically related groups, clonal complexes (CC), including those with special epidemiological significance called hyperinvasive clonal complexes. It is still unknown why some of them may persist for decades, whereas other are quickly replaced and disappear. As a consequence, the epidemiological situation of IMD is variable worldwide and greatly depends on the emergence and widespread of clones belonging to hyperinvasive clonal complexes. Their occurrence has serious implications for health policy, requiring often mass immunization campaigns. Paradoxically, alarming situations caused by hyperinvasive CCs stimulated the development and introduction of new vaccines against meningococci. Despite the unquestionable success of these vaccines, isolates of hyperinvasive clones constitute a permanent public health threat, because they are constantly circulating and able to modify their antigenic profiles to escape the host immune response. Therefore, continuous monitoring of meningococcal isolates including thorough molecular typing is indispensable and fundamental for taking appropriate preventive measures.

Key words: *Neisseria meningitidis,* clonal complex, epidemic, invasive meningococcal disease (IMD), meningococcal epidemiology

Introduction

Neisseria meningitidis (meningococcus, Men) is a gram-negative diplococcus carried as a commensal in the upper respiratory tract (nasopharynx) of an average of 8% to 25% humans who are the only known reservoir and host of this bacterium (Stephens, 2009). It is also known as a dangerous and devastating human pathogen, which can cross the mucosal surface, enter the bloodstream and multiply, leading to rapidly progressing septicemia or translocate across the blood-brain barrier causing meningitis. These severe infections called invasive meningococcal disease (IMD) are one of the major public-health threats worldwide (Stephens *et al.*, 2007).

The basic classification of meningococci is based on structural differences of the polysaccharide capsule, which is the major antigen linked to meningococcal virulence. Twelve serogroups have been distinguished and 6 of them (A, B, C, W, X, Y) are responsible for the majority of IMD worldwide. Meningococcal infections usually develop rapidly and at the beginning can be difficult to distinguish from other less severe febrile illnesses. General case fatality rate (CFR) is high, 9–12% and up to 40% in septicemia (Rosenstein *et al.*, 2001). Death can occur within a few hours from the first appearance of symptoms, and those who survive may suffer from permanent tissue damage, amputations, deafness and psycho-neurological sequelae. That is why rapid diagnosis, targeted antibiotic therapy and chemoprophylaxis are so crucial (Rosenstein *et al.*, 2001; Stephens, 2009). However, due to the very rapid and dramatic course of infection and often lack of time to administer proper treatment, it seems indisputable that the most effective way to prevent IMD is immunoprophylaxis. Commercially available polysaccharide vaccines protect against infections caused by meningococci of serogroup A, C, W and Y. The polysaccharide of serogroup B (MenB) is poorly immunogenic and homologous to human neural tissue. Consequently, vaccine studies against MenB have been focused on protein antigens and have led to the development of *e.g.* outer membrane vesicles (OMV) vaccines (Caesar

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et al., 2013; Panatto *et al.*, 2013; Vipond *et al.*, 2012) and others like Bexsero®, recently licensed in Europe (EMA, 2013) and in the USA (FDA, 2015) or Trumenba® up to date in the USA only (FDA, 2014).

Meningococcal virulence

Although many components and mechanisms associated with meningococcal virulence have been studied, the pathogenesis of IMD is still not fully understood. There are several bacterial factors like capsule, outer membrane components including *i.e.* pili, lipooligosaccharide (LOS) and outer membrane proteins (OMPs) which are associated with adhesion, transmission and invasion of meningococci. The key factor linked to meningococcal virulence is a polysaccharide capsule determining the serogroup. It has antiadherent properties affecting transmission and protects bacteria from phagocytosis, opsonization and complement-dependent bacteriolysis during invasion of host cells. It is also the major antigen of meningococci (except MenB) leading to a rise of bactericidal antibodies. Taking these into account and the fact that antigens A, C, W and Y have been predominant among meningococci, they have been used in polysaccharide and/or conjugate vaccines. Among other meningococcal factors which promote adherence to host cells, are pili and opacity proteins (*i.e.* Opa, Opc). Lipooligosaccharide is also involved in adherence and is known for its strong endotoxic activity. Porins such as PorA, PorB allow the passage of ions across the cell membrane. They modulate apoptosis and influence host immune response. Because they induce bactericidal antibodies during meningococcal disease, they have been used as vaccine antigens. Additionally, differences in porins composition are the basis of identification of serotype (PorB) and serosubtype (PorA). Proteins associated with iron acquisition *e.g.* FetA are also related to virulence and like porins are the target for bactericidal antibodies. In recent years many other outer membrane proteins have been identified, which are involved in the pathogenesis of IMD. Some of them became already, whereas others are still promising vaccine candidates. (Hill *et al.*, 2010; Tzeng and Stephens, 2000). Expression of the above mentioned components is dependent on many mechanisms. For example, the ability of meningococci to exchange genetic material responsible for capsule synthesis may cause modification of the capsule and change of serogroup, called capsule switching (Swartley, 1997). This property applies also to surface protein antigens like *e.g.* PorA, PorB, FetA (antigenic swift). Other mechanisms lead to on/off expression (phase variability) and concerns *i.e.* the capsule and LPS. Consequently, meningococci can become unrecognizable by the host and may escape from the immune response (Hill *et al.*, 2010, Tzeng and Stephens, 2000).

It is for sure that the course of infection depends not only on a bacteria's ability to invade, but also is host and environmental dependent. Factors that predispose to IMD are *i.e.* the lack of protective bactericidal antibodies, defects in the terminal complement pathway (C5-C9), the lack of properdin, immune suppression associated with splenectomy, nephritic syndrome or hypogammaglobulinemia. Viral upper respiratory tract infections as well as active and passive smoking are associated with injury of respiratory mucosa, which is a barrier to invasion, therefore its damage increases the risk of bacterial transmission and IMD. Transmission is also simplified by close contact with patient and crowding conditions *e.g.* in dormitory, military base, social events (Rosenstein *et al.*, 2001, Tzeng and Stephens, 2000).

Epidemiology of IMD

IMD may occur sporadically, but also in outbreaks, epidemics, and pandemics. The incidence of IMD is geographically variable and age-specific. The overall incidence is from less than 0.5 cases/100 000 to 1000 cases/100 000 population during epidemics in the sub-Saharan African countries (so-called "meningitis belt") (Halperin *et al.*, 2012). The incidence is the highest in children under 5 (especially in infants), teenagers and young adults (Rosenstein *et al.*, 2001; Stephens, 2009).

Distribution of serogroups varies globally, and has been changing during past decades. Meningococci of serogroups B and C (MenC) have been predominant in Europe, Australia and the Americas. Both have been responsible for sporadic cases, but significantly MenC are related to multiple outbreaks and epidemics whereas MenB to threatening hyperendemic situations/prolonged epidemics. Meningococci of serogroup A (MenA) have been the cause of large, seasonal epidemics in Africa and were also prevailing in Asia while in other continents they are very rare (Halperin *et al.*, 2012; Harrison *et al.*, 2009). In Africa, meningococci of serogroup X have been also reported to cause large outbreaks between 2006 and 2010 (Jafri *et al.*, 2013). Meningococci with capsule antigen Y are responsible for one third of IMD cases in the USA. Interestingly, they were rarely observed in Europe in the past, while recently noteworthy increase in the Scandinavian countries have been observed (Bröker *et al.*, 2014; Harrison *et al.*, 2009). Epidemic potential of serogroup W meningococci (MenW) associated generally with very sporadic IMD, was recognized during two epidemics started in Saudi Arabia during Hajj Pilgrimages and a large epidemic in Burkina Faso (Harrison *et al.*, 2009; Jafri *et al.*, 2013).

Molecular typing

To know and understand the epidemiology of IMD and why some isolates are more virulent than others, profound epidemiological analysis of cases including typing of isolates is needed. In the past, phenotype characteristics based on determination of serogroup: serotype:serosubtype (defined by capsule:PorB:PorA, respectively *e.g.* C:2a:P1.5,2) was used but because of lack, poor or masked expression of surface antigens, not all isolates could be typed. In contrast, molecular methods based on PCR and DNA sequencing, provide typing data always when a gene is present, even without protein expression. Additionally, most DNA-based methods can be directly used on clinical samples, when a culture is negative due to *e.g.* early antibiotic treatment. These techniques have higher discriminatory power and generally enable distinction between isolates responsible for outbreaks and sporadic cases. Currently, a widely used method in molecular typing is multilocus sequence typing (MLST). This technique was introduced for the first time in 1998, for meningococci and later adapted for other bacterial species. MLST is based on the sequencing of internal fragments of seven housekeeping genes whose alleles combinations determine sequence type (ST). Among meningococcal numerous STs, there are groups of epidemiologically significant and related STs called clonal complexes (CC). Each CC has one central ST, with the exception of 41/44CC which has two. Isolates that have at least four out of seven loci identical with central ST are considered to be related and form CC (Brehony *et al*., 2007; Maiden *et al*., 1998). At present, more than 11 000 sequence types are determined and grouped into 46 clonal complexes whereas these without relatedness to other STs were designated as singletons (http://pubmlst.org/neisseria/, Brehony *et al*., 2007). Despite large variability, there is a limited number of CCs characterised by increased propensity to cause invasive disease or epidemics. They are called hyperinvasive clonal complexes and are responsible for the majority of meningococcal infection in the world. Despite their deep analysis including genome sequencing in comparison to carriage strains, the reasons of their superiority and predominance are still not fully understood (Schoen *et al*., 2014)

The most common hyperinvasive clonal complexes responsible for IMD – historical background and the present status

Some hyperinvasive CCs have been persistent for many decades, other emerged recently or were prevalent in the past and nowadays are observed very rarely. In spite of this variability, among all hyperinvasive

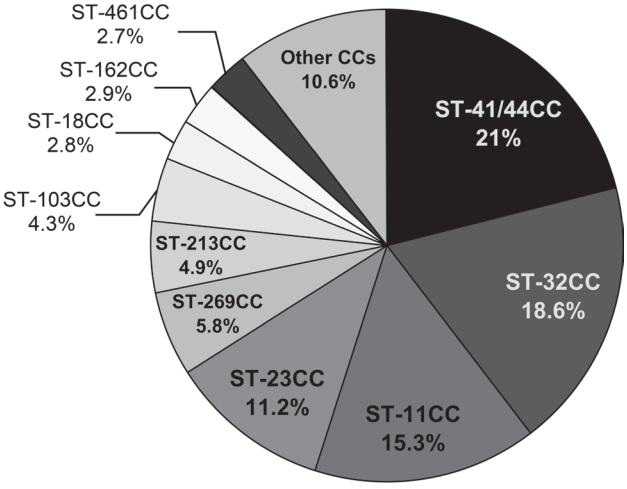

Fig. 1. Distribution of MLST clonal complexes (CCs) among *N. meningitidis* isolates responsible for IMD in Europe in 2011 (in accordance to Whittaker, 2013).

CCs the most frequently mentioned in the literature and overrepresented among meningococci are those belonging to ST-5CC, ST-32CC, ST-41/44CC and ST-11CC. European data confirmed that in 2011 meningococci of ST-41/44CC were the most common, followed by ST-32CC and ST-11CC and their percentage was estimated as high as 21%, 18.6% and 15.3%, respectively (Fig. 1.; Whittaker, 2013). Isolates of the four mentioned CCs have been associated in the past with large epidemics, numerous outbreaks, temporary/geographical incidence increase or long-lasting hyperendemic situations/prolonged epidemics. Their emergence and expansion required public health investigations and targeted interventions, like mass vaccination campaigns using available vaccines but also had an indisputable influence on the construction of new ones. (Table I) (Read, 2014; Watkins and Maiden, 2012).

ST-5CC

ST-5 clonal complex is represented by meningococci of serogroup A. Isolates belonging to this CC were in the past the cause of a few pandemics and since 80s have been predominant in Asia and especially in the "meningitis belt" comprising countries in Sahelian and sub-Sahelian Africa. These isolates have been devastating for Africa, being responsible for thousands of IMD cases during yearly epidemics starting with the dry season and cyclical large epidemics occurring every 5–10 years; for example in 1996 there were 150 000 cases and 16 000 deaths reported (Caugant and Nicolas, 2007; Achtman, 1997; Nicolas *et al*., 2005, Zhang *et al*., 2008). The extremely high number of infections and deaths as well as insufficient impact of polysaccharide vaccines, have led to initiate in 2001 the "Meningitis Vaccine

Table I
Epidemiological events with ST-5, ST-41/44, ST-32, ST-11 clonal complexes and their influence on vaccines development

Clonal complex	The most common serogroup/ phenotype	Selected epidemiological occurrences	Time period	Vaccine used/developed	References
ST-5CC	Serogroup A	3 pandemics, all started in China and spread: 1. to Russia, Scandinavia and Brazil; 2. to Nepal, India, European countries, the USA and Africa; 3. to Mongolia and Moscow yearly epidemics during dry seasons and cyclical wide epidemics every 5–10 years with incidence rate reaching 1000/100 000	 60s 80s 90s Since 80s	polysaccharide vaccines used in massive immunization campaigns since 2010 conjugate vaccine, MenAfriVac® in national immunization programmes	Achtman, 1997, Caugant and Nicolas, 2007, Nicolas *et al.*, 2005 www. meningvax. org
ST-41/ 44CC	B:4:P1.7–2,4	hyperendemic situation in New Zealand	90s–2000s	OMV vaccine MeNZB®	Holst *et al.*, 2013 Martin and McDowell, 2004
ST-32CC	B:15:P1.7,16	hyperendemic situation/prolonged outbreaks in • Norway • Oregon, the USA	 60s and 70s 1990–2000s	OMV vaccine MenBvac®	Harrison *et al.*, 2009
	B:14:P1.7,16	• Normandy, France	2000s		Holst *et al.*, 2009
	B:4:P19,15	hyperendemic situation/prolonged outbreaks in • Spain • Cuba, Brazil and South Africa	 70s 80s	OMV vaccine VA-MENGO-BC®	Pannato *et al.*, 2013 Racloz and Luis, 2010
	B:15:P1.3	outbreaks in Chile	80s	OMV vaccine WRAIRc®	
ST-11CC	Serogroup C	outbreaks and increase of IMD incidence in Canada, the USA, Israel, Czech Republic, Iceland, Finland, Norway, the UK, Greece, Spain and Australia. further widespread all over the world	Since mid-80s	initially polysaccharide vaccines used in massive immunization campaigns conjugate vaccines against MenC (MCC) in national immunization programmes in many European countries and Americas	Campbell *et al.*, 2009 Halperin *et al.*, 2012 Harrison *et al.*, 2010 Jafri *et al.*, 2013 Miller *et al.*, 2002
	Serogroup W	international epidemics associated with the Hajj Pilgrimage to Mecca, when epidemic clone was transferred into 16 countries *i.e.* the USA, the UK, Nordic countries, Europe wave of epidemics in Burkina Faso with app. 12 000 cases	2000, 2001 2002	polysaccharide vaccines used in massive immunization campaigns	Hahné *et al.*, 2002, Harrison *et al.*, 2009 Mayer *et al.*, 2002, Taha *et al.*, 2000

Project", which resulted in developing an accessible, inexpensive conjugate polysaccharide vaccine against serogroup A for Africa, MenAfriVac® (www.meningvax.org). The vaccine was introduced in December 2010, first in Burkina Faso (Djingarey *et al.*, 2012) and in accordance with the last data more than 217 milion people in 15 countries have been vaccinated so far. The effects of mass vaccinations achieved so far are very promising. Decreased number of cases associated with serogroup A as well as sharp decline of the total incidence of IMD has been noticed (Daugla *et al.*, 2014; Novak *et al.*, 2012; www.meningvax.org) and MenA has disappeared from the "vaccinated" countries of the "meningitis belt" (Table I) (Caesar *et al.*, 2013).

ST-41/44CC and ST-32CC

Nowadays ST-41/44CC is the most common CC among MenB in Europe representing 31.7%. Its members are observed not only among MenB but also MenC and non-groupable meningococci (Whittaker, 2013). For example in Poland in the period 2009–2011 this CC was the third and the second most common CC among meningococci of serogroup B and C, respectively (Skoczyńska *et al.*, 2013). According to the MLST database, ST-41/44CC is very diverse, containing the highest number of STs among hypervirulent clonal complexes. At the time of writing as many as 1800 sequence types have been assigned to this CC

while ST-5CC, ST-32CC or ST-11CC include 53, 597 and 359 STs, respectively (http://pubmlst.org/neisseria/). At present, members of this complex are circulating worldwide, being responsible mainly for sporadic cases, but in the past it was different. For example in New Zealand, a clone of ST-41/44CC with phenotype B:4:P7-2,4 caused a threatening hyperendemic situation (Watkins and Maiden, 2012). A yearly increase in the number of IMD cases (from 53 in 1990 to 650 in 2001) forced the development of an OMV vaccine containing protein antigens of epidemic clone in order to stop its further expansion (Martin and McDowell, 2004). The vaccine proved to be effective, decreasing IMD incidence from 24.7/100 000 in 2001 to 2.6/100 000 in 2008 (Table I) (Holst *et al*., 2013). Taking this success into consideration as well as the clone with phenotype B:P7-2,4 worldwide distribution and OMVs adjuvant properties, vesicles from MeNZB® vaccine have been used recently in a new 4-component vaccine 4CMenB, Bexsero®. The vaccine contains also other protein antigens like factor H-binding protein (fHbp), neisserial heparin-binding antigen (NHBA) and neisserial adhesin A (NadA) identified by reverse vaccinology (Caesar *et al.,* 2013; Pannato *et al*., 2013; Serruto *et al*., 2012). Analysis of MenB conducted in many countries showed high predicted coverage by the 4CMenB vaccine and it is believed that this vaccine will be efficacious against the majority of serogroup B meningococci (Vogel *et al*., 2013).

Second, the most common CC among MenB is ST-32CC. In 2011 meningococci B/ST-32CC represented 27.8% of MenB responsible for IMD in Europe while in Poland between 2009–2011 ST-32CC meningococci were predominant and represented 32.4% of MenB (Skoczyńska *et al*, 2013; Whittaker, 2013). ST-32CC isolates have been related mainly to sporadic IMD. However, in the past, as already mentioned Men-41/44CC, meningococci of this complex were the cause of few prolonged epidemics. The first report of epidemic potential of ST-32CC came from Norway, from 1969. Then its spread throughout European countries, South Africa and the Americas was observed (Harrison *et al*., 2009; Harrison *et al*., 2010; Racloz and Luiz, 2010). According to the MLST database and phenotyping analysis, three epidemic clones were characterized. To control these strain-specific serogroup B epidemics, three OMV vaccines were developed (Table I). Although their safety and effectiveness was proven and a significant decrease in the incidence of IMD was observed, for years they were used for protection against strains that were used to construct the vaccines only. Later on, their utility against strains sharing some antigens with vaccine strain was proven, as was shown in Normandy (Caron *et al*., 2011; Holst *et al*., 2009; Pannato *et al*., 2013).

ST-11CC

Meningococci belonging to ST-11CC have had a significant impact on global meningococcal epidemiology and advances in strategies to control and prevent IMD. In contrast to other clonal complexes, ST-11CC isolates have been present among meningococci of several serogroups including B, C, W and Y (http://pubmlst.org/neisseria/, Barroso *et al*., 2013), although the majority represent MenC. In Europe in 2011 60% of MenC belonged to ST-11CC whereas in Poland in the period 2009–2011 only 11.3% (Skoczyńska *et al*., 2013; Whittaker, 2013). They are well known for their unique epidemic potential. In the mid-1980s and 1990s several local outbreaks as well as an increase in IMD incidence caused by MenC/ST-11CC occurred in some provinces of Canada. It is threatening that at the same time ST-11 widely spread into other countries on all continents (Table I) (Ashton *et al*., 1991; Gottfredsson *et al*., 2006; Harrison *et al*., 2010; Jelfs *et al*., 2000; Kremastinou *et al*., 1999; Krizova and Musilek, 1995; Miller *et al*., 2001; Tribe *et al*., 2002). During the following years a next wave of IMD caused by ST-11 clone in Canada was observed (Tsang *et al*., 2004; Zhou *et al*., 2012) and isolates belonging to this CC have become predominant among MenC all over the world causing more numerous outbreaks and clusters *i.e.* in educational institutions, military barracks, or other crowded, semi-closed places. (Bijlsma *et al*., 2014; Brehony *et al*., 2007; Chacon-Cruz *et al*., 2014; Deghmane *et al*., 2010; de Lemos *et al*., 2007; ECDC, 2013; Fazio *et al*., 2009; Garnier *et al*., 2011; Simon *et al*., 2013). Also in Poland between 2006–2009 several outbreaks were recorded as well as an increase of incidence of MenC ST-11CC (Fig. 2) (Grecki and Bienias, 2006; Kadłubowski *et al*., 2007; Skoczynska *et al*., 2010; Waśko *et al*., 2009).

It is necessary to emphasize that infections caused by ST-11CC meningococci of serogroup C are associated with significantly higher CFR and sequelae rates in comparison to isolates of other CCs. This can be due to the fact that ST-11CC isolates more frequently cause septicemia than meningitis and mortality from septicemia is generally higher (Rosenstein *et al*., 2001). It was also interesting that outbreaks caused by the mentioned clone affected mainly teenagers and young adults (Ashton *et al*., 1991; Jensen *et al*., 2003; Krizova and Musilek, 1995; Skoczynska *et al*., 2013; Smith *et al*., 2006; Trotter *et al*., 2002). Because of frequent outbreaks occurrence, significant increase in incidence and higher mortality, massive immunization campaigns were carried out. Initially, available polysaccharide vaccines were widely used, for example in Canada and Czech Republic (De Wals *et al*., 1996; Kriz *et al*., 1995). Alarming changes in epidemiology accelerated the development of conjugate vaccines against

Fig. 2. Map of Poland showing regions where seven outbreaks (I–VII) caused by ST-11CC isolates between 2006 and 2009 took place (in the brackets is the number of reported cases).

MenC (MCC), rapidly introduced for the first time in 1999 in the UK. It is interesting and unique that their introduction was initiated without any evidence of the vaccines' efficacy (it was extrapolated from polysaccharide vaccines), only with safety and immunogenicity confirmation (Miller *et al*., 2001). MCC vaccines apart from dramatic decrease of the incidence of MenC IMD in vaccinees, reduced also nasopharyngeal carriage, resulted in desirable herd immunity effect (Campbell *et al*., 2009; Larrauri *et al*., 2005; Miller *et al*., 2001). Consequently, the vaccine was successively introduced into national immunization programmes in some other European countries as well as in Australia, Canada and South America (Halperin *et al*., 2012; Jafri *et al*., 2013).

Although ST-11 clone of serogroup C was the cause of many outbreaks and epidemics all over the world, the largest epidemics were caused by another variant of ST-11 meningococci, possessing W polysaccharide capsule. The first two international epidemics took place in 2000 and 2001, and were associated with the Hajj Pilgrimage to Mecca. Asymptomatic carrier pilgrims returned to the countries of their origin and transferred epidemic clone to their relatives/contacts in few continents (Hahné *et al*., 2002; Mayer *et al*., 2002; Taha *et al*., 2000). Later, in 2002, a wave of epidemics was also reported in Burkina Faso (Table I) (Harrison *et al*., 2009). Currently changes in the distribution of W/ST-11 clone have been observed *e.g.* after the introduction of a conjugate vaccine against MenA in some African countries like Burkina Faso, where the clone has become predominant (Hossain *et al*., 2013; MacNeil *et al*., 2014). Spread and/or a noticeable increase of cases caused by the clone has been noted recently also in Europe, South America and Asia (Barra *et al*., 2013; Barroso *et al*., 2013; Ladhani *et al*., 2014; Yamamoto *et al*., 2013; Zhou *et al*., 2013).

Concluding remarks. It is still unknown why some meningococcal clones may persist for decades and are characterized by unusual high virulence or epidemic potential, including sudden, drastic increase of incidence, hyperendemic IMD, and outbreaks/epidemics, whereas others are quickly replaced and disappear. As a consequence, the epidemiological situation of IMD is dynamic and variable worldwide and greatly depends on the emergence and widespread of clones belong-

ing to hyperinvasive clonal complexes as *e.g.* ST-5CC, ST-11CC, ST-32CC and ST-41/44CC. Their occurrence has serious implications for health policy, often requiring mass immunization campaigns. Paradoxically, alarming situations caused by the mentioned CCs have stimulated the development and introduction of new vaccines, counting OMV vaccines against few epidemic clones, different conjugates against serogroups A, C, W, Y meningococci and recent protein vaccines targeting mostly MenB. Experience gained during mass vaccinations has confirmed that immunoprophylaxis is the best and the most effective means to control IMD. Despite the unquestionable success of vaccination, isolates of hyperinvasive clones constitute a continuous public health threat, because they are constantly circulating and able to modify their antigenic profiles to escape host immune response. Therefore, continuous monitoring of meningococcal isolates including thorough molecular typing is indispensable and fundamental for taking appropriate preventive measures.

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