Consensus paper

Hexavalent vaccines: characteristics of available products and practical considerations from a panel of Italian experts

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Keywords

Hexavalent vaccines • Combination vaccines • Vaccine manufacturing • Safety • Co-administration

Summary

Combination vaccines represent a valuable technological innovation in the field of infectious disease prevention and public health, because of their great health and economic value from the individual, societal, and healthcare system perspectives.
In order to increase parents' and healthcare professionals' confidence in the vaccination programs and maintain their benefits to society, more information about the benefits of innovative vaccination tools such as combination vaccines is needed.
Purpose of this work is an examination of available hexavalent vaccines, that protect against Diphtheria, Tetanus, Pertussis, Poliomyelitis, Hepatitis B and Haemophilus influenzae type b infections. From the epidemiological updates of vaccine preventable diseases to the vaccine development cycle, from the immunogenicity of antigenic components to the safety and co-administration with other vaccines, several aspects of available hexavalent vaccines are discussed and deepened.
Also a number of practical considerations on schedules, age of employment, strategies for vaccination recovery, vaccination in at-risk births are issued, based on the recommendations of Italian Ministry of Health, Italian Society of Pharmacology (SIF), Italian Society for Pediatrics (SIP), Italian Federation of Family Paediatricians (FIMP) and Italian Society of Hygiene, Preventive Medicine and Public Health (SItI).

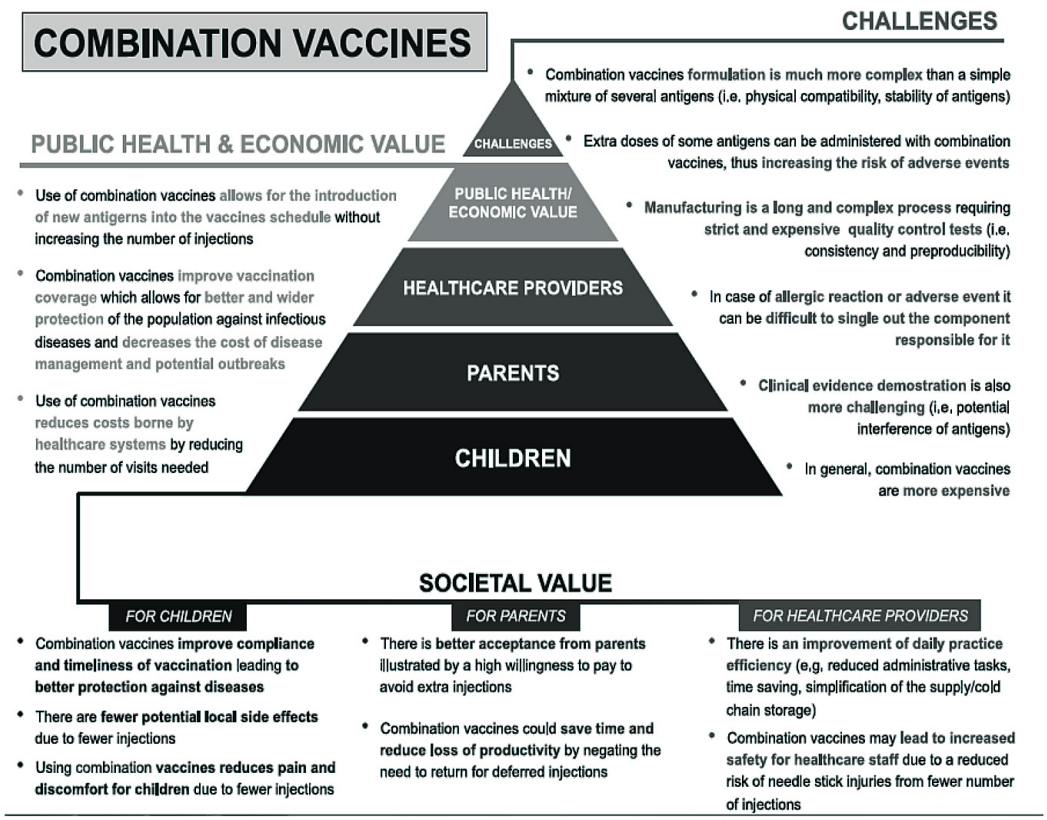
Introduction

Since the very early vaccines formulation, increasingly important targets have been reached in prevention area. Nowadays, vaccinations are one of the most relevant resource in Public Health, providing prevention against diseases once cause of epidemics [1].
Recently, thanks to combination vaccines usage, other important targets have been achieved. In fact, combination vaccines ensure in a single injection a multiple immunization [2]. Reduction in the number of administrations leads to a decrease in ambulatory accesses and to a better safety profile of vaccinations programs, given the fact that a significant proportion of adverse events following immunization (AEFI) results from the act of injection. Combination vaccines availability is therefore an important tool in achieving a safe and successful protection against numerous pathogens, simplifying prospective introduction of new vaccines in a less crowded vaccine schedule [3, 4].
Combination vaccines usage provides a relevant value to health, with positive effects in the people health with a social and economic saving (Fig. 1) [5].
Purpose of this work is an examination of hexavalent vaccines, that protect against Diphtheria, Tetanus, Pertussis, Poliomyelitis, Hepatitis B and *Haemophilus influenzae* type b (Hib) infections.

Hexavalent vaccines development cycle: from manufacturing to delivery

Hexavalent vaccines development is time-consuming and complex, and passes through the production of singles vaccines providing Diphtheria, Tetanus and Pertussis antigens (dTaP). Several quality controls are applied in every development cycle phase, so that efficacy and safety profiles have been raising over the years. The first phase is 12-years-long on average and includes the pre-clinical and clinical stages, the regulatory agencies

Fig. 1. Combination vaccines usage: a public health, economic and social value.



registration, and the discharge of the first approved and deliverable vaccine batch; within this time vaccine formulation are continually tested in order to provide the definitive composition and to develop adequate production systems. The second phase is no more than 36-months-long for the hexavalent vaccines development, and includes the production stage; up to 70% of this time is devoted to quality controls; the remaining time is required for antigens, carriers and adjuvants processing and combination, to the achievement of the hexavalent composition. Hence the hexavalent vaccine is in accordance with the highest standards in pharmaceutical industry, in terms of efficacy and safety [6, 7].

During the vaccine development cycle, that is long and elaborate, it's possible a vaccine batch doesn't pass quality control check; this may due, for example, to inadequate antigenic concentrations or to an unstable formulation. In this scenario, the whole hexavalent combination is stopped and not utilized, even if the quality control check failure is related only to a single antigen. Hence, it could be necessary a 2-years-long time to restore the production standards, with the consequence of supplies delivery deficiencies. Pharmaceutical factories are dealing with those challenges: if we consider the production stages timing, it's necessary that supplies variations and vaccines demands will ideally be made with a 3-years advance in order to maintain an adequate availability [6, 7].

Details about antigens preparation processing and timing, and about their formulation in the hexavalent vaccines, are described in Figure 2.

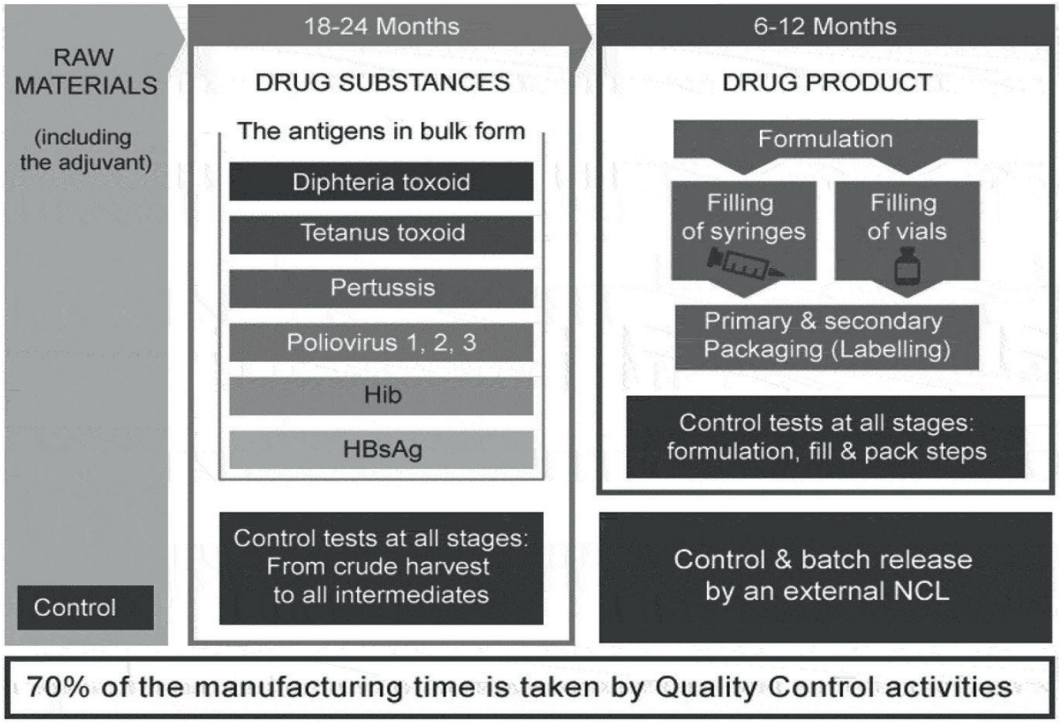
In addition to quality assessment, complexity and heterogeneity of different regulatory systems amongst countries is another relevant challenge in vaccines production, since it dictates another time delay to the final vaccines availability. Particularly, marketing and import authorization of vaccines lots are main critical issues in this context [6, 7].

Hexavalent vaccine preventable diseases: epidemiological notions

Due to vaccinations introduction, a considerable number of diseases have been controlled and avoided. Those diseases comprehend Diphtheria, Tetanus, Pertussis, Poliomyelitis, Hepatitis B and Hib infections, for whom immunization can be reached in a single vaccine administration, hexavalent vaccine [8].

In order to get the best control and prevention of four of these diseases (Diphtheria, Tetanus, Pertussis and Poliomyelitis), booster vaccinations are necessary, as

Fig. 2. Quality controls performed within the different hexavalent vaccines production stages.



required by 2017-2019 Italian National Vaccine Prevention Plan (PNPV) [9]: (i) dTaP-IPV vaccination (pediatric vaccine with a full antigenic amount) in 6-years-old children; (ii) dTap-IPV vaccination (adult vaccine with a low antigenic amount) in 12 to 19-years-old subjects and (iii) dTap vaccination (adult vaccine with a low antigenic amount) every 10 years in adult age.
Thanks to such strategies, a significant reduction in epidemiological trends of these diseases have been reached (Tab. I).

Diphtheria

An increasing reduction in Diphtheria cases in Europe, from 42/100000 population in 2008 to less than 0.1/100000 population in 2015, is documented in last ECDC Report [10]. Two cases of Diphtheria were nonetheless reported in Spain and Belgium [11].
In Italy, Diphtheria incidence had a dramatically reduction, up to 0 events in the 2010-2013 period. In the 2015-2017 period, nonetheless, 8 Diphtheria cases were reported. In particular, one C. Diphtheriae strain was a toxin-producer, responsible of cutaneous diphtheria, while other cases were caused by a non-toxin producer strain [12].
Even in countries where diphtheria is not endemic, thanks to high vaccination coverages, risk should not be underestimated. Some C. Diphtheriae toxin-gene-free strains can colonize nasopharynx and then cause pharyngitis, bacteraemias, endocarditis, septic arthritis, abscesses and pneumonia. The presence of non-toxigenic

Tab. I. Italian epidemiology of hexavalent vaccine preventable diseases: a comparison before and after vaccine introduction [16].

Disease	Notified case number (on average) every year, before vaccinations	Notified case number (on average) in the period 2010-2013	% Reduction
Diphtheria	7000	0	100%
Tetanus	700	60	– 91.4%
Pertussis	21000	509	– 97.6%
Poliomyelitis	2000	0	100%
Hepatitis B	3000	419	– 86.0%
Haemophilus influenzae type b	69	6	– 91.3%

C. Diphtheriae strains has been recently documented in United States and in Europe, Italy included. Uncommonly, some of these strains are armed with toxin genes and could start producing the toxin through spontaneous reversion to toxigen strain or homologous recombination among different corynebacteriophages [13, 14].

Tetanus

Even though Tetanus is a preventable disease, several cases occur in Italy each year, with the highest notification and hospitalization rates compared to other European countries and other high-income countries. In 2014, in the last ECDC Report, 84 events were reported in Europe, of which 48 confirmed in the laboratory. 35 events (45% of total cases) were reported in Italy, with an incidence of 0.02/100000 population [15].
In 2010-2013 period, 60 cases/year (with 20 deaths/year) were notified in Italy. Mostly belonged to unvaccinated elderly or to elderly that did not performed booster, with a reduction of 91.4% compared to pre-vaccine era [16]. Lack of pediatric vaccination or booster dosages has been related to the higher disease incidence in population aged > 64 years. Anti-Tetanus vaccination basal cycle is followed by multiple boosters to the adult age, when a booster dosage must be repeated every 10 years. The importance of boosters against Tetanus is due to antibodies levels decline over years and to the time needed for antibodies production in the period between infection and death [17].
In 2001-2010 period 2 cases of pediatric Tetanus (age < 14 years) were notified; successively no pediatric cases were reported until June 2017, when 2 cases were notified, the first in Sardinia and the second in Piedmont; both child were not immunized [18].

Pertussis

Pertussis is a very contagious disease that can occur in every ages, but it's more serious in newborns and infants [19]. Within this age, hospitalization rates due to complications as apnea, seizures and pulmonary hypertension are higher; fatality rate can reach 1%. Complications and death risks are higher in pre-term born compared to term-born [20, 21].
Currently, Pertussis is the vaccine-preventable infectious disease most frequent in high-income countries. Its real impact is hardly evaluable and scarcely perceived by population and even by health workers [22].
From 1999 to 2009 in Italy a decrease in Pertussis cases trend was reported, thanks to high vaccine coverage. However, Pertussis is probably underestimated, especially in adolescents and young adults, due to the milder clinical presentation and the scarce usage of laboratory testing. Among those subjects, parents represent the main source of infection for children, in which the disease can be more severe [23, 24].
In Italy, about 500 cases/year have been notified from 2011 to 2015 [25]. Hence, compared to the pre-vaccine era, Pertussis incidence has a 97.6% reduction; this result was also achieved thanks to hexavalent vaccine containing Pertussis antigens (Tab. I).

Since both natural and vaccine-acquired immunization period against Pertussis is time-limited, it's pivotal to per-form booster dosages in the two cases. In fact, although clinical manifestations are milder as individuals grow, every subject can infect newborns, in a time before their acquired immunity reaches protective levels. Consequently, as planned in the PNPV paper, it's essential to provide a booster with DTaP-IPV in pre-school age, a booster with dTap-IPV in adolescence age and a booster with dTap every 10 years in adult age [26].
Cocoon strategy was proposed in order to reduce newborns infections. It consists of a booster administration in every potential newborn contacts.
Recent recommendations made by Italian experts, in accordance with WHO, and based on scientific studies, demonstrate that the best cost-effective intervention in newborns Pertussis prevention is the vaccination with dTap vaccine in the pregnant woman, ideally in the third trimester: doing so, maternal antibodies will be transmitted to the fetus and can protect the newborn in the window time from the birth to the first vaccination [27].

Poliomyelitis

Poliomyelitis (polio) is an infectious disease burdened with a severe prognosis. The main risk in polio is a nonreversible flaccid paralysis, affecting especially pediatric population. The sole option to prevent Poliomyelitis consequences is vaccination. The Global Polio Eradication Initiative contributed to reduce over 99% of the global Poliomyelitis incidence. Europe was declared polio-free in 2002. The last case of polio was reported in Turkey in 1978, while 3 cases of polio were reported in Bulgaria in 2001, belonging to Rom children from India; in that last context, autochthonous transmission was stopped [28].
In Italy, thanks to vaccination (mandatory from 1966), the last Poliomyelitis case was reported in 1982. Still, in order to reach Poliomyelitis eradication, it's pivotal to carry on the IPV (Inactivated Poliovirus Vaccine) vaccination program, since as long as a single infected child is present, every child in the world is potentially susceptible [29-31]. In fact, even now it's important to maintain high antibodies levels since the risk of importation, and the consequent transmission, is present: Poliomyelitis is still endemic in Afghanistan and Pakistan [32].

Hepatitis B

Worldwide, 257 million people are Hepatitis B virus (HBV) chronic carriers. Chronicity risk is greater especially if the HBV infection is precocious: risk rate is 90% in birth-infected children, 30-50% in infected children less than 4 years old, 1-10% in the population of age > 4 years [33].
Most European countries provide vaccination against HBV infection. Italy was a paradigm since in 1991 it established anti-HBV vaccination as mandatory for every new born and for 12-years-old children [34, 35].
Trends' analysis concerning case numbers in the period 1985-2016 shows a significant decline from 1991. Inci-

dence decline was related especially to age 15-24 years, due to newborns and 12-years-old children vaccination [36]. Thanks to this intervention, Hepatitis B incidence had a 86% reduction and chronic carriers prevalence had a reduction from 3% to less than 1% [16, 37]. This decline is extremely important, since Hepatitis B is one of the leading cause of liver cirrhosis and liver cancer, whose diagnosis is 10 years late after infection. At a distance of 20 years from the introduction of the anti-HBV vaccination, a significant decline in the case numbers of Hepatitis B associated liver cirrhosis and liver cancer is evident [38].
Over the past five years, 19% of HBV acute infections regarded non-Italian subjects arriving from HBV endemic regions, as East Europe (9% of overall HBV notified cases to the Italian Integrated Epidemiological System of Acute Viral Hepatitis [SEIEVA]) and Africa (4,9% of cases) [39].

Haemophilus influenzae type b

Hib could be cause of severe and invasive diseases as meningitis, sepsis, pneumonia. Hib epidemiology is not easy to define, since in several cases a timely laboratory confirmation is not made [40]. In 2007-2014 period 0.6 cases/100000 population/year were notified in Europe. Most of these cases was related to younger subjects: incidence was 23.4 cases/100000 newborns [41]. In Italy, the introduction of the vaccination against Hib led to 91.3% disease incidence reduction compared to pre-vaccine era. Also hospitalization rates due to Hib-disease halved compared to pre-vaccine era, as described in a recent Italian study [42]. Nevertheless, concomitantly with vaccination coverage decline, severe invasive Hib disease occurred recently in children less than 2 years old [42-44].

Currently available hexavalent vaccines

The first combined vaccines were bivalent, with Diphtheria and Tetanus antigen (DT o dT); later Pertussis antigens were included thus forming the trivalent DTwP and dTaP vaccines, containing the inactivated Bordetella Pertussis and acellular antigenic components. Hexavalent combined vaccines, obtained with the antigens mentioned above and in addition polio, Hepatitis B, Hib antigens, are available for over 15 years. In Italy, as other countries in Europe, hexavalent vaccines are the most frequently used vaccine for the immunization of infants and toddlers against Diphtheria, Tetanus, Pertussis, Hepatitis B, Poliomyelitis and disease caused by Hib [45, 49].
Currently in Italy, three hexavalent combination vaccines are available: Infanrix Hexa®, used since 2000; Hexyon®, since 2013; and Vaxelis®, recently authorized. Their characteristics, described in the Summary of Product Characteristics (SPC) documents, are summarized in Table II [46-48].

Immunogenicity of antigenic components: focus on Pertussis, Hepatitis B, Hib

Although different in composition, the three vaccines have comparable immune response, resulting protective against the 6 target diseases. The immunogenicity of hexavalent vaccines has been extensively studied and comparative clinical trials have been carried out: in particular, the studies conducted with the 2 + 1 schedule reproduce a vaccination scheme similar to the one in current vaccination schedule of the PNPV, both for the age of use both for the co-administration with anti-pneumococcal and anti-rotavirus vaccines [49, 50].
In these studies, the responses to each antigen were evaluated using pre-established standard seroprotection correlates as concentration and antibody titers to be achieved in order to assert that the vaccine has determined antibody protection (seroprotection).
In the case of Pertussis antigens, for which a seroprotection indicator is not available, the response to the vaccine is evaluated considering the concentrations of specific antibodies produced after vaccination that are higher than those present before vaccination.
The results demonstrate the high immunogenicity of all the antigens included in the three hexavalent products, with high percentages of children with seroprotection and similar values for each antibody response. Specifically, the percentage of seroprotected children who responded to the hexavalent vaccines was non-inferior for all anti-body concentrations compared to those of children immunized with monovalent or fewer components (e.g. dTaP) [3, 4].
Also clinical data of safety, immune response and effectiveness are available. The results of the follow-up of clinical studies confirmed the presence of protective antibody titers. Effective protection up to the age of the dTaP-IPV booster has been demonstrated, for Hepatitis B antibody titers were at protective levels up to the age of pre-adolescence, guaranteeing protection against the risk of transmission in adolescents and adults. In addition, epidemiological surveillance programs conducted in several countries such as Sweden, Denmark and Germany, on pathologies such as Pertussis and Hib, have confirmed the effectiveness of hexavalent vaccines.
These information are reported in the SPC of the two long-standing hexavalents authorized, Infanrix Hexa® and Hexyon®, and will be available in a few years for Vaxelis®, that have the most recent authorization.

Pertussis

All vaccines combined with acellular components (aP acellular Pertussis) contain Pertussis toxoid (PT). The other antigenic components of Bordetella Pertussis, sometimes included, are: filamentous haemagglutinin (FHA), pertactin (PRN) and fimbriae types 2 and 3 (FIM). Pertussis vaccines are different in the formulation, combination and concentration in micrograms of the individual components, and also in the production methods used, such as the detoxification and purification. Therefore, the comparison between different aP vaccines cannot be based only on the number of antigen-

Tab. II. Summary of currently available hexavalent vaccines characteristics.

	Infanrix Hexa® [46]	Hexyon® [47]	Vaxelis® [48]
Hib -PRP	10 µg Conjugated to Tetanus toxoid	12 µg Conjugated to Tetanus toxoid	3 µg Conjugated to Meningococcal protein
Pertussis	PT 25 µg FHA 25 µg PRN 8 µg	PT 25 µg FHA 25 µg	PT 20 µg FHA 20 µg; PRN3 µg FIM type 2,3: 5 µg
Diphtheria toxoid	Not less than 30 UI *mean value	Not less than 20 UI *lower limit CI 95%	Not less than 20 UI *lower limit CI 95%
Tetanus toxoid	Not less than 40 UI	Not less than 40 UI	Not less than 40 UI
IPV Polio	Poliovirus inactivated type 1, 2, 3	Poliovirus inactivated type 1, 2, 3	Poliovirus inactivated type 1, 2, 3
Hepatitis B - HBsAg produced in	Saccharomyces cerevisiae	Hansenula polymorpha	Saccharomyces cerevisiae
Ready to use	No	Yes	Yes
Co-administration	Yes	Yes	Yes
Preterm	Yes	Yes	Yes
Minimum age	Not specified	6 weeks	6 weeks
Maximum age	No limits	No limits	No limits
Follow-up studies of antibodies persistence	Yes	Yes	Not available
Effectiveness results	Yes	Yes	Not available

ic components contained, also because the contribution to immune protection by each anti-gen is not completely clear [26].

The essential component is PT, present in all aP vaccines, and directly responsible for the development of a protective immune response following vaccination.

The FHA it is the antigen that over time is less mutated genetically, unlike the PRN whose mutations led to the diffusion of pertactin-resistant strains.

There is no evidence on the contribution of immune protection given by the fimbriae in the newborn, while it seems they may play a role when contained in the booster vaccines for the adult [26].

Moreover, it is well known that even the natural infection with Pertussis, which obviously contains "all the components", is able to induce permanent immunity [24, 26].

Although no Pertussis or serologic protection indicator is available for Pertussis, all aP vaccine antigens present in hexavalent vaccines have shown high immunogenicity in comparative clinical studies (both between hexavalent and both combined vaccines with fewer components) in terms of the presence of higher antibody levels after vaccination compared to the pre-vaccination serological test [51].

Several evidences have clearly shown how prevention and control of Pertussis are based on the adoption of a vaccination schedule that includes, in addition to primary vaccination in the newborn, booster in childhood, adolescence and adulthood (every 10 years), and on reaching and maintaining high coverage, regardless of the employed aP vaccine and the number of components contained. In particular, in Denmark, where an aP vaccine with only PT component has been used for over 15 years, the disease has been well controlled and no outbreaks have occurred [52, 53]. Also in Sweden, after 19 years of epidemiological surveillance, Pertussis is effectively controlled throughout the nation, regardless of the type of vaccine used, with one or more components [54].

As underlined by the WHO Pertussis technical group (WHO SAGE Pertussis Working Group), the key point in the control of the pathology is the achievement of high vaccination coverage and a schedule with adequate timing for the childhood and booster in adulthood [55]. The same consideration are described by the American Society of Pediatrics and the CDC in the 12th edition of the Pink Book, as well as in the last WHO Pertussis Vaccines Position paper 2015: a summary of recommendations and evidences of use of the aP vaccines is reported in Table III.

Hepatitis B

The current Hepatitis B vaccines, both monovalent and combined, contain the HBV virus surface antigen (HBs), produced in yeast cells by recombinant DNA technology. In the case of hexavalent vaccines, the yeast cells used are Hansenula polimorpha and Saccharomyces cerevisiae: all HBs antigens have been shown to be highly immunogenic, although the production processes differ in the cell line used.

In the registered clinical trials, high seroprotection rates were detected in vaccinated children, with overlapping results in the comparison between hexavalent vaccines and monovalent anti-Hepatitis B vaccine (anti-HBs titre ≥ 10 mIU/mL) [49, 50]. Furthermore, in the clinical trials follow-up up to pre-adolescence age, anti-Hepatitis B antibodies result at highly protective levels in response to the administration of a challenge dose, with important implications for long-term memory and protection against possible future infections [46, 47, 59]. Further

studies on vaccinations for Hepatitis B have shown that, after the primary vaccination during the first year of life, protective antibody levels are maintained until adolescence age. Therefore, thanks to the vaccines used up to now, including the hexavalents, it is not necessary to administer a booster for Hepatitis B in the general population, while a booster dose may be necessary in those at risk and in non-responders [60, 61].

Haemophilus influenzae type b

In the currently available hexavalent vaccines registration studies, the immunogenicity of the Hib vaccine was assessed by measuring serum IgG antibodies against the PRP capsular antigen. The thresholds set for short- and long-term protection are, respectively, ≥ 0.15 μg/mL and ≥ 1 μg/mL. Considering these as reference values, the responses against the PRP antigen of Hib have recorded high levels of seroprotection in children vaccinated with the hexavalents in use [49, 50].
Furthermore, the efficacy of the Hib vaccine is supported by various evidences deriving from national surveillance systems that monitor the incidence of cases of Hib disease and evaluate the trends before and after introduction of vaccination. In Germany, a population-based system that integrated hospital admissions surveillance and molecular laboratory diagnosis allowed to assess the impact of anti-Hib vaccination after the introduction of tetravalent and pentavalent products, respectively in 1996 and 1998. The first surveillance data for the two-year period 1998-1999 showed that the number of cases of Hib disease in children aged 0-5 ranged from 28 to 13 [62]. Subsequently, data related to a longer period (August 2000 - December 2004) made it possible to estimate the efficacy of the vaccine in relation to the doses administered: the effectiveness of the Hib vaccine was 96.7% (95% CI: 87.7-99.1) for the cycle complete primary, and 98.5% (95% CI: 94.5 to 99.6) for the booster dose. Estimates of efficacy of the anti-Hib component in hexavalent vaccines did not show significant differences compared to combined tetra and pentavalent vaccines [63].
Regarding Italian region, before the introduction of universal vaccination against Hib, the incidence of invasive disease caused by Hib in children aged less than 5 years increased from 2.5/100.000 in 1994 to 4.5/100.000 in 1998, a trend most likely due to the implementation of active surveillance for the invasive disease from Hib based on laboratory data, implemented at that time in some Italian regions. Since 1999, after the introduction of anti-Hib vaccination with 2 + 1 schedule, an excellent control of the disease has been registered, underlined by the reduction in hospitalization rates due to invasive disease [42].

Co-administration with other vaccines

The safety and immunogenicity of hexavalent vaccines do not change significantly when co-administered with other vaccines included in the child's vaccination schedule [64]. Clinical studies of the three hexavalent products demonstrated elevated immunogenicity and safety standards of the 2 + 1 hexavalent schedule co-administered with anti-pneumococcal and anti-rotavirus vaccines [6, 48, 49]. Further clinical studies have also confirmed the co-administration with vaccines such as anti-meningococcal conjugate, anti-measles, mumps, rubella and anti-varicella vaccines. The indications for all possible co-administrations are reported in the relative SPC [46-48].
In general, as recommended in the CDC guide on co-administrations, all vaccines may be administered in the same session, without any limitation in the number of vaccines administered (alternating sites for subsequent injections), unless reported in SPC [65, 66].

Tab. III. Summary of recommendations and evidences of use of the aP vaccines.

Plotkin S et al. 2013 [56]	Surveillance programs demonstrate the efficacy of each aP vaccine in achieving excellent pPertussis control
WHO 2016 [57]	Long-term national surveillance studies conducted in Sweden and Denmark, where 10 2-component vaccines are also used, showed high levels of effectiveness in preventing Pertussis regardless of the antigenic content of the various aP vaccines used. All polyvalent aP vaccines showed high levels of effectiveness in preventing pertussis independently of aP in these contents
WHO SAGE Working Group 2014 [55]	There is no evidence to conclude that one type of aP vaccine is superior to others. The available data reinforce the importance of achieving and maintaining high coverage and implementing appropriate vaccination schedules
CDC Pink Book 2015 [19]	The efficacy of the vaccines aP it's variable between 80% and 85%, the respective confidence intervals of these overlap each other, suggesting that none of the aP vaccines is significantly more effective than other
American Academy of Pediatrics 1997 [58]	Although the different aP vaccines available differ in their formulation of Pertussis antigens, their efficacy is similar
Gabutti et al. 2015 [26]	The use of the current polyvalent vaccines with aP allowed the achievement and maintenance of high vaccine coverage that, regardless of the type of vaccine and the number of AP in these contents, is the key factor for successful vaccination interventions against Pertussis

Safety of hexavalent vaccines

Vaccines can be considered among the most controlled and safe pharmaceutical products. Before marketing authorization and introduction into immunization programs, they are subjected to different stages of safety and efficacy assessment. Once authorized, the production processes are subject to accurate and continuous checks and the presumed adverse events are constantly monitored and analyzed, in order to guarantee to the population safe and high quality vaccines. Furthermore, the production of vaccines is controlled in compliance with standards indicated by international organizations such as the European Medicines Agency (EMA) and the WHO.

Although the vaccines currently used in immunization programs are safe and effective, they, like all drugs, are not exempt from possible adverse events, although rare, after vaccination. An AEFI is defined as any adverse clinical event that occurs following the administration of a vaccine and which does not necessarily have a causal relationship with the use of the vaccine [67].

The results of the safety reports analyzes collected in the clinical studies on hexavalent products showed good tolerability of these vaccines, confirmed both by the study follow-up and by the phase IV post-marketing surveillance systems. The results of the safety of hexavalent vaccines are included in the relative SPC [46-48].

In general, a higher but not clinically significant rate of fever and local symptoms (from mild to moderate, and in any case transient) was recorded compared to vaccines with fewer components. However, the use of combined hexavalent vaccines is overall safer because, by subjecting the child to a single injection instead of six, the total frequency of these reactions is reduced, which also occurs for co-administration with other vaccines for children [5, 68, 69].

Detailed data on post-marketing surveillance of vaccines in Italy derive from the latest AIFA report, which summarizes the post-marketing surveillance activities on vaccines conducted in Italy in 2016 [70]. Reports of suspected ad-verse reactions to hexavalent vaccine, collected through the National Pharmacovigilance Network in 2016 were 1'127, of which 670 (59.4%) occurred in the period from January 1 to December 31, 2016. The serious reactions were 188, 16.7% of suspected reports included in the National Pharmacovigilance Network. Most reports (No. 845, 75%) refer to the simultaneous administration of hexavalent and other vaccines (in particular the pneumococcal vaccine), following the co-administration provided by the vaccination schedule.

As with other vaccines, most reactions are mild and transient. The ten reactions more frequently reported after hexavalent vaccine in 2016 in Italy are described in Table IV.

As regard to Sudden Infant Death Syndrome (SIDS), the Report states that there is no evidence of causal relationship between exposure to vaccines and SIDS, and that the incidence of this is the same both in the presence and both in the absence of vaccination. A short distance between the SIDS and the vaccination does not imply, therefore, any cause-effect relationship.

Further support for the safe use of vaccines is the Guide to contraindications to vaccinations, including the hexavalent one, in which the real contraindications are distinguished from the false ones [71] (Tab. V).

Practical considerations

Summary of Product Characteristics: clarifications on terminology

The terminology used in the summary of product characteristics, that is sometimes a source of interpretative confusion, has been recently deepened and clarified in a document of the "Calendario per la Vita" Italian board, a panel of experts belonging to the main scientific society engaged in vaccination and public health [72].

One of the key points is the paragraph 4.1 which establishes indications of the use of the vaccine, even with a medical point of view. Section 4.2 indicates the dosage and method of administration of the vaccines. The other sections contain specifications on population groups in which efficacy and safety studies were performed, data on interactions with other drugs (section 4.5) and pharmacodynamic properties (section 5.1). The information contained in these paragraphs should not be confused with the indications of the vaccine1. For example, with regard to the age of employment, hexavalent vaccines are given "starting at 6 weeks of life", without an upper limit of use. Considering that they contain a dose of "pediatric" antigens, even if this indication is not included in the technical data sheet, their use is recommended up to 7 years of age. Obviously, both the vaccines and the drugs used in the therapeutic field are usually studied in the age groups where their greatest use is expected. However, the fact that a vaccine has safety studies up to 24/36 months, for example, does not preclude its use in older age groups, as it is also reported in the indications for hexavalent vaccines and appropriately specified also by the European Medicines Agency (EMA).

Tab. IV. First ten reactions reported in order of frequency after administration of hexavalent vaccine in 2016 in Italy.

Type of reaction	Frequency (No.)
Fever	618
Hyperpyrexia	146
Cry	124
Irritability	100
Drowsiness	91
Swallow at the time of vaccination	55
Erythema at the time of vaccination	51
Pain at the time of vaccination	49
Agitation	41
Diarrhea	40

Tab. V. Contraindication (true or false) and precautions for the use of hexavalent vaccine.

Contraindications	Precautions	False contraindications
Severe allergic reaction (anaphylaxis) after administration of a previous dose Severe allergic reaction (anaphylaxis) to a component of the vaccine *Temporary contraindications* Encephalopathy not attributable to another cause within seven days of administration of a previous dose of hexavalent until clarification of the cause or stable disease	Encephalopathies and encephalopathies with seizure of unknown aetiology, including West syndrome Guillain-Barré Syndrome and related syndromes within 6 weeks of administration of a previous dose of vaccine Severe or moderate acute illness, with or without fever Peripheral neuritis after the administration of a previous dose Immediate generalized urticaria after the administration of a previous dose Extreme prematurity Severe latex allergic reaction (for products containing latex in the syringe) Immunocomplexing (arthus) after administration of a previous dose	Positive anamnesis for febrile seizure Neurological disorders (well-controlled seizures, cerebral palsy, developmental delay) Episode of hypotonia-hyporesponsiveness within 48 hours after administration of a previous dose of hexavalent Hyperpyrexia after a previous dose of hexavalent Persistent and uncontrolled crying for more than 3 hours after previous administration of hexavalent Family history of SIDS Non-extreme prematurity History of local reaction extended after previous dose Clinical history of pertussis Family history of convulsions Family history of adverse events after administration of aP or wP

A further example regards the employment in particular groups of children, such as preterm births. In this case, although there is no specific indication in paragraph 4.1, the administrability is confirmed both by the absence of relative contraindications (section 4.3), and by the presence of specific precautions regarding very premature births (section 4.4).
Furthermore, as indicated by AIFA in the Drugs Information Bulletin, the use in clinical practice of drugs, or even vaccines, already registered but used in a manner not in accordance with the summary of the characteristics of the drug is defined "off-label" authorized product [73]. Therefore, also taking into account the current legislation that regulates the off-label use of medical products and compliance with the authorized therapeutic indications (Article 3 of Legislative Decree 17 February 1998, converted into Law of 8 April 1998), we can conclude that all and three hexavalent vaccines can be used up to 7 years of age and can be used in premature babies [74]. In essence, the use of hexavalents in premature babies and in all subjects up to 7 years is to be considered as "on-label" use, adequate to what is prescribed in the summary of product characteristics.

Schedule and specific dosage

The results obtained in the studies conducted with 2 + 1 schedule, with administrations at the 3rd, 5th and 11th -13th month of age, showed that the antibody responses reach high levels of seroprotection for all the antigens of the three hexavalents [49, 50].
The two long-standing hexavalents authorized and used with 2 + 1 schedule have been shown to prevent the six diseases for which they determine immunity, feedback obtained from the follow-up of clinical studies and the anal-ysis of epidemiological surveillance programs [46-48].

Age of employment and recovery of defaulters

All hexavalent vaccines are indicated in all children from 6 weeks of age and, as described in the summary of product characteristics, there is no limit of upper age. The recommendations of the "Calendario per la Vita" board for the recovery of vaccinations in non-compliant children have been reiterated by the Ministry of Health: the use of hexavalent vaccines is recommended up to 7 years of age. Both the board and the Ministry of Health recommend to the healthcare workers to propose to the parents, as the first choice, the administration of the hexavalent vaccine, as it allows to reduce to a minimum the number of sessions and the number of administrations and to minimize the possible side effects [72, 75].
The recommendations of the board and the Ministry of Health are in line with those contained in the vaccination position paper of the Italian Society of Pharmacology (SIF), drawn up together with the Italian Society of Pediatrics (SIP), with the Italian Society of Preventive Medicine and Public Health (SITI), wih the Federation of Family Pediatricians (FIMP) and with the General Practitioners (FIMMG). The document, approved by the National Institute of Health, recommends the use of vaccines containing antigens in pediatric concentration up to 7 years, such as hexavalent vaccines.
The ECDC and the WHO also recommend the use of pediatric vaccines, such as hexavalents, in older children, in line with EMA indications on these type of vaccines [76, 77].
Therefore, the use of hexavalent vaccines is supported by: (i) the evidence of the registration studies (efficacy and safety studies) conducted in the population groups that include the age groups in which their greatest use is expected; (ii) the experience of vaccines combined with similar formulation, such as tetravalents, already studied and indicated up to higher ages (DTaP-IPV up to 12 years of age); (iii) pharmacovigilance studies and

post-marketing surveillance; (iv) the recommendations of scientific societies, international organizations and the Ministry of Health.

Vaccination in preterm births

The WHO defines as a preterm the child born before the 37th week of gestation; moreover, prematurity is distinguished in mild, medium and severe according to the gestational age at birth [78].
In the vaccination schedule of the PNPV, no difference is made between term and premature births, indicating that all children are vaccinated with hexavalent from the 3rd month of life [9].
The results of a recent literature review have confirmed that all monovalent and combined vaccinations give sufficient protection guarantee when administered in preterm births, with the only exception of the monovalent Hepatitis B vaccine which, when given at birth (born from HBsAg positive mothers), gives a lower immune response and should be repeated at one month of life to obtain adequate protection. Sufficient and protective anti-HBs concentrations are produced by preterm infants at the completion of the hexavalent vaccination schedule at 9-12 months of age [79].
Furthermore, the results of epidemiological surveillance of invasive Hib disease in Tuscany in 2007-2017, the period in which Infanrix Hexa® was first used and after Hexyon® in all children, including preterm births, have shown that both hexavalent vaccines are safe and effective, since no case of illness was registered among the children vaccinated in the study period (except for a child with a congenital antibody defect, unable to produce antibodies to protective levels) [80].
The three hexavalent vaccines do not have a specific indication in point 4.1 of the summary of product characteristics relating to preterm births. Infanrix Hexa® and Vaxelis® summary of product characteristics report clinical data on premature delivery, although on a limited number of newborns. Hexyon® does not have specific data on preterm births in the data sheet, but data on use and effectiveness were collected in an epidemiological surveillance program in Tuscany.
As proof of the fact that the use of the three hexavalent vaccines fully respect the authorized therapeutic indications, the summary of products characteristics of the three vaccines show the same information on the "very preterm" born in point 4.4 (Special warnings and precautions for use), born before the 28th week and with a history of respiratory failure. In these children, in fact, considering the potential risk of apnoea onset, it is necessary to monitor the respiration in the hospital for 48-72 hours after administration. Because the benefit of vaccination is high in this group of newborns, vaccination should not be stopped or postponed [46-48].
These precautions are valid for all pediatric vaccines that can be administered in newborns and for which preterm use, including very premature ones, is also expected.
Therefore, all hexavalent vaccines can be used in preterm births, with a 2 + 1 schedule and respecting the same timing of term births, without delaying immunization.

Vaccination in the births of a positive HBsAg mother

The available hexavalent vaccines report in the summary of product characteristics the possibility of use in children born to HBsAg positive mothers [46-48]. The 2017-2019 PNPV schedule includes monovalent vaccination for Hepatitis B at birth and at 30 days of life. In these children, the vaccination series continues with the 2 + 1 schedule of the hexavalent [9]. As demonstrated by a review of clinical trials, this scheme ensures the production of protective antibody concentrations in all children, including preterm births [79].

Interchangeability

Given the existence of combined vaccines containing slightly different components, practitioners often ask them-selves how to continue the cycle if the previous vaccine given is unknown or is no longer available at the time of the next dose.
In general, it is preferable to continue the vaccination schedule with the same product with which immunization was started [81]. Although it is possible, if not explicitly contraindicated in the data sheet, to use a hexavalent vaccine different from that used in the previous dose of the schedule, it is appropriate that safety and efficacy data are available, and that this mode of use is described in summary of products characteristics [5, 46-48].
Actually, only the hexavalent Hexyon® possesses these requirements, and the related methods of use are described in summary of products characteristics.

Preparation method: pre-filled syringe or to be reconstituted

While Hexyon® and Vaxelis® are available in fully-liquid formulation, in pre-filled and ready-to-use syringe, Infanrix Hexa® requires, prior to the administration, the reconstitution in the main syringe of the Hib antigen, contained in-stead in a vial [46-48].
Several studies have compared these types of vaccines, with results of a reduction of about 5 times the risk of possible errors in the preparation and halving of the time of administration for those in formulation in pre-filled syringe compared to vaccines that require a reconstitution process [82-85].

Conclusions

In consideration of what has been described and evoked by the literature and by the registration studies of the hexavalent vaccines, together with the recent recommendations on their use of the "Calendario per la Vita" board, it can be concluded that: (i) combined vaccines reduce the number of administrations and therefore the frequency of local reactions to the injection site and of

crying, as well as reducing the number of visits and access necessary for completing the vaccination schedule; (ii) clinical studies have shown that the three hexavalent vaccines have a high immunogenicity and safety profile; (iii) hexavalent vaccines may be co-administered in the same vaccination session with pneumococcal and anti-rotavirus vaccines, as foreseen in the PNPV calendar; (iv) hexavalent vaccines can be administered in preterm births with a 2 + 1 schedule, without delaying the start of the immunization cycle; moreover, for the very preterm births (ie born before the 28th week of gestation) and with respiratory insufficiency, the precautions for use described in the summary of product characteristics must be followed; (v) two hexavalent vaccines have antibody persistence data in summary of product characteristics, demonstrated by follow-up of clinical trials (up to 9-11 years for anti-HBs antibodies) and efficacy data from epidemiological surveillance programs. For the third vaccine, with the most recent authorization, the same data will be available in a few years; (vi) all hexavalents are highly effective in preventing Pertussis, as demonstrated by related surveillance programs; the vaccination of the mother during pregnancy is the most effective intervention for the prevention of the disease in the first months of life; (vii) there are no differences in immunogenicity between different antigen formulations; (viii) it is preferable to continue the schedule with the same vaccine with which it was started, or that specific modalities are reported in the summary of product characteristics; (ix) the formulation in pre-filled syringe reduces the risk of possible errors and time of preparation and administration.

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Authors' contributions

All Authors have made a substantial contribution to the conception, design, analysis and interpretation of data, drafting the article and revising it critically for intellectual content; all Authors approve the final version submitted to the *Journal of Preventive Medicine and Hygiene*.

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