



New vaccine introductions in Africa before and during the decade of vaccines – Are we making progress?



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ABSTRACT

Vaccines are excellent investments with far-reaching rewards beyond individual and population health, but their introduction into national programs has been historically slow in Africa. We provide an overview of the introduction of new and underutilized vaccines in countries of the WHO African Region by 2017, using data from the WHO-UNICEF Joint Reporting Form. By 2017, all 47 countries had introduced vaccines containing hepatitis B (compared to 11% in 2000 and 98% in 2010) and *Haemophilus influenzae* type b (Hib) (compared to 4% in 2000 and 91% in 2010). The proportion of countries that had introduced other vaccines by 2017 was 83% for pneumococcal conjugate vaccine (PCV) from 7% in 2010, 72% for rotavirus vaccine from 2% in 2010, 55% for the second dose of a measles-containing vaccine (MCV2) (compared to 11% in 2000 and 17% in 2010), and 45% for rubella-containing vaccines (RCV) (compared to 4% in 2000 and 7% in 2010). From 2000 to 2010, there was no significant difference between countries eligible (N = 36) and those not eligible (N = 10) for Gavi support in the introduction of hepatitis B and PCV. However, Gavi eligible countries were more likely to introduce Hib and non-Gavi eligible countries were more likely to introduce MCV2 and RCV. From 2010 to 2017, the only significant differences that remained between the two groups of countries were with mumps, inactivated polio and seasonal influenza vaccines; which non-Gavi eligible countries were more likely to have introduced. There has been significant progress in the introduction of new childhood vaccines in Africa, mostly driven by Gavi support. As many countries are expected to transition out of Gavi support soon, there is need for countries in the region to identify predictable, reliable and sustainable immunization funding mechanisms. This requires commitments and actions that go beyond the purchase of vaccines.

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1. Introduction

Immunization is one of the most cost-effective public health interventions and remains one of the best buys in public health [1,2]. An extensive investment in smallpox vaccine eradicated the disease from the global scene at a cost of US\$ 100 million over a 10-year period, between 1967 and 1977 [2]. Global savings from smallpox eradication have exceeded US\$ 1.3 billion a year in treatment and prevention costs ever since [2]. Measles vaccination between 2000 and 2016 prevented 20.4 million global deaths,

making measles vaccine one of the greatest value investments for public health [3]. While they have been progress towards elimination of measles, the surge in measles outbreaks globally raises concerns of pushing back the won gains. Overall, the 6 basic antigens (bacille Calmette-Guérin, diphtheria, tetanus, pertussis, polio and measles) originally recommended as childhood vaccines when the Expanded Program on Immunization (EPI) was established in 1974 prevent more than two million child deaths each year [2]. By introducing and ensuring universal access to newer vaccines (including, but not limited to those against hepatitis B, *Haemophilus influenzae* type b (Hib), human papillomavirus, meningococcus serogroup A, pneumococcal disease, and rotavirus), over 25 million future deaths could be averted between 2011 and 2020 and billions of dollars in economic benefits could be generated by 2020

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[4]. In general, new vaccine introduction into the EPI in Africa has been slow [5,6]. Since the establishment of the EPI in 1974, more vaccines have been recommended for use in the childhood immunization program, but there has been a very slow uptake of these in the African region. Vaccines against hepatitis B and Hib took decades to be introduced in most African countries; after their introductions in high-income countries [6,7].

It is against this background that the Global Vaccine Action Plan (GVAP) 2011–2020 was initiated, to provide universal access to life saving vaccines [4]. The GVAP is a roadmap to achieve the vision of the Decade of Vaccines i.e. “a world in which all individuals and communities enjoy lives free of vaccine preventable diseases”. The GVAP has five strategic goals, one of which is to develop and introduce new and improved vaccines and technologies. One of the targets for this strategic goal is for all low-income and middle-income countries to introduce at least one new or underutilized vaccine by 2020 [4]. As the Decade of Vaccines (2011–2020) draws to a close, there is a need to evaluate the status of the introduction of new vaccines in low and middle-income countries. In this paper, we assessed the status of new vaccine introduction in the World Health Organization (WHO) African Region from 2010 to 2017 using the publicly available data from the WHO/UNICEF Joint Reporting Form (JRF) [8].

2. Method and analysis

We used data available in the WHO-UNICEF Joint Reporting Form (JRF) as of 31 December 2017. The JRF contains immunization data, including vaccine introductions, from all WHO Member States worldwide [8]. Data in the JRF is collected using standardized questionnaire completed by a national immunization authority of a reporting country [8]. The deadline for which the data must be completed is usually mid-April although countries may update or amend previously reported data at any time with prior permission from WHO/UNICEF [8]. The last updated JRF was on 18 September 2018. All the data reported is reviewed for quality, completeness and consistency before publication on the WHO website [8]. The data is categorical with countries responding “yes” if a vaccine is in the schedule and “no” if a vaccine is not in the schedule. The vaccine introduction data in the excel sheet for each country include the following variables: “vaccine in schedule”; “year of introduction in the entire country” and “year of introduction in part of the country”. We counted the number (and calculated the proportion) of countries that had each vaccine in their national schedule by 2000, 2010, and 2017. We used the chi-squared test to assess the differences between proportions using the Stata Software version 15.1. We defined statistical significance at the alpha

level of 5%. We did not require a formal ethical review for this study as the data are publicly available.

3. Results

There were 46 countries in the WHO African Region from 2000 to 2010. With the independence of South Sudan in 2011, the number of countries went up to 47. Of the 47 countries, 37 (79%) were eligible for support from Gavi, the Vaccine Alliance, and 10 (21%) were not eligible. To be eligible for Gavi support, countries must have a Gross National Income (GNI) per capita of US\$ 1580 or below on average over the past three years. Although Angola and the Republic of Congo have transitioned out of Gavi support, we considered the two countries as Gavi eligible for the purposes of this paper because they received Gavi support to introduce the vaccines considered in the paper. The 10 countries not eligible for Gavi support (self-financing) for the whole study period from 2000 to 2017 include Algeria, Botswana, Cape Verde, Equatorial Guinea, Gabon, Mauritius, Namibia, Seychelles, South Africa and Swaziland.

Table 1 shows the status of introduction of new and underutilized vaccines (NUVs) in WHO African countries between 2000 and 2010 while Table 2 shows the status of introduction between 2010 and 2017. Between 2000 and 2010, hepatitis B and Hib were the most introduced underutilized vaccines to the basic six antigens of the EPI. In 2010, an average of 3 of the 12 NUVs had been introduced by most countries; this increased to an average of 7 of the 12 NUVs introduced by most countries in 2017. The three most frequently introduced NUVs were for hepatitis B, Hib and yellow fever; and four vaccines introduced in 2017: PCV, rotavirus, IPV or bivalent oral polio and second dose of measles containing vaccines. In 2010, self-financing countries introduced the most NUVs. By 2010, Seychelles had introduced 7 NUVs while Mauritius and South Africa had introduced 6 NUVs. The Gambia was the only Gavi eligible country that had introduced 6 NUVs in 2010. In 2017, Seychelles and Mauritius had 10 and 11 NUVs respectively. The introduction of NUVs in Gambia and South Africa increased from 6 in both countries to 9 in 2017.

In 2010, 45 of 47 countries reported to have introduced hepatitis B vaccine. By 2017 all the countries had introduced hepatitis B vaccine with Equatorial Guinea and South Sudan introducing the vaccine in 2013 and 2014 respectively. Of 47 countries administering hepatitis B vaccine in 2017, 37 were Gavi-eligible countries and 10 were self-financing countries. Hib vaccine was in the schedule in 43 countries in 2010 and this increased to 47 by 2016. Botswana, Equatorial Guinea, Nigeria and South Sudan are among countries that introduced Hib vaccine after 2010. There were 35 Gavi eligible

Table 1
Comparison of vaccine introductions in Gavi and non-Gavi countries in Africa between 2000 and 2010.

Antigen	Introductions in Gavi		Introductions in non-Gavi		P value
	2000 (N = 36)	2010 (N = 36)	2000 (N = 10)	2010 (N = 10)	
Hepatitis B	2 (6%)	36 (100%)	5 (50%)	9 (90%)	0.217
<i>Haemophilus influenzae</i> type b	1 (3%)	35 (97%)	1 (10%)	8 (80%)	0.115
Pneumococcal conjugate	N/A	2 (6%)	0 (0%)	1 (10%)	0.530
Rotavirus	N/A	0 (0%)	0 (0%)	1 (10%)	–
Measles second dose	1 (3%)	1 (3%)	4 (40%)	6 (60%)	0.000
Rubella	0 (0%)	1 (3%)	1 (10%)	2 (20%)	0.115
Human papillomavirus	N/A	0 (0%)	0 (0%)	0 (0%)	–
Mumps	0 (0%)	0 (0%)	2 (20%)	3 (30%)	0.008
Influenza	0 (0%)	0 (0%)	0 (0%)	0 (0%)	–
Inactivated polio vaccine	0 (0%)	0 (0%)	0 (0%)	1 (10%)	0.217

N refers to number of countries. Values provided are absolute counts (percentage), unless otherwise indicated. Yellow fever and meningococcal A conjugate vaccines are not included in the table because these are not recommended for all countries in the region.

The p-value shows the Fisher exact test results for difference in introductions between Gavi and self-financing countries by 2010.

Table 2

Comparison of vaccine introductions in Gavi and self-financing countries in Africa between 2010 and 2017.

Antigen	Introductions in Gavi		Introductions in non-Gavi		P value
	2010 (N = 36)	2017 (N = 37)	2010 (N = 10)	2017 (N = 10)	
Hepatitis B	36 (100%)	37 (100%)	9 (90%)	10 (100%)	–
<i>Haemophilus influenzae</i> type b	35 (97%)	37 (100%)	8 (80%)	10 (100%)	–
Pneumococcal conjugate	2 (6%)	33 (86%)	1 (10%)	6 (60%)	0.051
Rotavirus	0 (0%)	28 (76%)	1 (10%)	6 (60%)	0.272
Measles second dose	1 (3%)	18 (49%)	6 (60%)	8 (80%)	0.077
Rubella	1 (3%)	14 (38%)	2 (20%)	7 (70%)	0.073
Human papillomavirus	0 (0%)	3 (8%)	0 (0%)	4 (40%)	0.029
Mumps	0 (0%)	0 (0%)	3 (30%)	4 (40%)	0.001
Influenza	0 (0%)	0 (0%)	0 (0%)	4 (40%)	0.001
Inactivated polio vaccine	0 (0%)	27 (73%)	1 (10%)	10 (100%)	0.067

N refers to number of countries. Values provided are absolute counts (percentage), unless otherwise indicated. Yellow fever and meningococcal A conjugate vaccines are not included in the table because these are not recommended for all countries in the region.

The p-value shows the Fisher exact test results for difference in introductions between Gavi and self-financing countries by 2017.

and 8 self-financing countries that introduced Hib vaccine in 2010, this increased to 37 and 10, respectively in 2016.

In 2010, only 2 Gavi eligible countries (Gambia and Rwanda) and one self-financing country (South Africa) had introduced pneumococcal conjugate vaccine (PCV). This number increased to 39 countries in 2017. Six (6) of the 39 countries who had introduced PCV were self-financing countries. Of those that introduced PCV in 2017, Nigeria was the only country to partially introduce the PCV vaccine with plans to roll-out to the entire country.

In 2010, 7 countries reported to have introduced measles-containing-vaccine second-dose, this increased to 26 countries in 2017. Eighteen of these countries including South Sudan were Gavi eligible and 8 were self-financing countries. There was an increase in rubella vaccine introduction from 3 in 2010 to 21 in 2017. Seychelles was the first country in Africa to have introduced rubella vaccine in 1980 followed by Mauritius in 1996 and Cabo Verde in 2010. There were 14 Gavi eligible and 7 self-financing countries that introduced rubella vaccine in 2017.

There were 3 self-financing countries that had introduced mumps vaccine by 2010, this increased to 4 self-financing countries in 2017. The first country in the WHO African region to introduce mumps vaccine was Seychelles in 1998 followed by Mauritius in 2000 and Cabo Verde in 2010. Algeria introduced mumps vaccine in 2017.

Among the 28 countries in the WHO African region identified to be at risk of yellow fever (YF), only 23 risk countries had introduced the yellow fever vaccine in 2017. Burundi, Ethiopia, Mauritania, South Sudan and Uganda are classified as at risk, but had not introduced YF vaccine as of 2017. Seychelles and Sao Tome and Principe, although not identified at risk of YF, had introduced the vaccine in 1995 and 2003 respectively making total of 25 countries in the region that had introduced YF in

2017. Equatorial Guinea was the only country to introduce the YF vaccine in 2017. Of the 25 countries that had introduced YF vaccine by 2017, three were self-financing countries (Equatorial Guinea, Gabon and Seychelles). Kenya remains the only country at a higher risk to YF in 2017 that has not introduced the vaccine in the entire country.

The region had a sharp increase in rotavirus vaccine introduction from one country in 2010 to 34 countries in 2017. Of the 34 countries to introduce the vaccine by 2017, 28 were Gavi eligible countries and 6 were self-financing countries. South Africa was the only self-financing country in the region to introduce rotavirus vaccine by 2010, first launched in one district in 2008 followed by a national roll out in 2009 (Table 1).

In 2017, 7 countries in the region had introduced human papilloma virus vaccine nationwide. Of these, four (Botswana, Mauritius, Seychelles and South Africa) were self-financing countries.

None of the countries in the WHO African region had introduced meningococcal vaccine (MenAfriVac) in 2010 as MenAfriVac only was licensed for use in 2010. Six Gavi supported countries, including Burkina Faso, Central African Republic, Chad, Ghana, Mali and Niger had introduced meningococcal vaccine by 2017.

Four (4) self-financing countries, Algeria, South Africa, Mauritius and Swaziland had introduced seasonal influenza vaccine by 2017. None of the countries in the region had introduced seasonal influenza vaccine in 2010.

Only South Africa had introduced inactivated polio vaccine (IPV) in 2010, in 2017 37 countries had introduced the vaccine. The majority of countries in the region to introduce IPV were Gavi eligible (27 countries) compared to self-financing countries (10 countries). Countries that are still to introduce IPV by 2017 include Burkina Faso, Eritrea, Ghana, Malawi, Rwanda, Sierra Leone, Togo, United Republic of Tanzania, Zambia and Zimbabwe.

Table 3

When vaccines first become licensed, first country to license and first pre-qualified by the WHO.

Antigen	Year of First License	First Country to License	First Year of WHO Prequalification
Hepatitis B	1986	United States	1987
<i>Haemophilus influenzae</i> type b	1987	United States	1997
Pneumococcal Conjugate	2000	United States	2000
Measles	1963	United States	1993
Rubella	1969	United States	2006
Mumps	1963	United States	–
Yellow fever	1953	United States	1987
Rotavirus	1998	United States	2007
Human Papillomavirus	2006	China	2009
Influenza (seasonal)	1945	United States	2009
Polio Vaccine – Inactivated	1955	United States	1997

We tested our hypothesis, whether a Gavi eligible country was more likely to introduce the NUVs. We found no significant statistical relationship ($p > 0.05$) to conclude that Gavi eligible countries were more likely to introduce the 10 vaccines introduced between 2010 and 2017. The introduction of three vaccines in this period; mumps ($p < 0.001$), human papilloma virus vaccine ($p = 0.029$) and seasonal influenza ($p < 0.001$) were strongly associated with non-Gavi support (Table 2). In Table 1, between 2000 and 2010, mumps ($p < 0.008$) and MCV2 ($p < 0.000$) were strongly associated with self-financing countries. The list of vaccines when first become licensed and pre-qualified are shown in Table 3. A complete list of the vaccines when it was prequalified by the WHO can be found on the WHO website https://extranet.who.int/gavi/PQ_Web/.

4. Discussion

The aim of the study was to examine the progress made in the introduction of new and underutilized vaccines (NUVs) between 2000 and 2017 in the WHO African region. We found significant progress in the introduction of NUVs across the WHO African region (Tables 1 and 2). An average of 7 NUVs were introduced in the African region by 2017 compared to 3 in 2010. One of goals of the Decade of Vaccines (DoV) 2011–2020 is to ensure that all low and middle-income countries introduce one or more appropriate NUVs by 2020 [4]. That goal has since been achieved although coverage needs to be raised for these vaccines.

By 2017, all countries had introduced hepatitis B and haemophilus influenzae type b (Hib) vaccines thereby demonstrating that the introduction of NUVs in the WHO African region is achievable. While this is a measure of success, it took 17–20 years for all countries in the WHO African region to introduce, two highly effective low-cost vaccines, hepatitis B and Hib. Compared to well-developed countries, this is a lag period of over 30 years for many countries, between the time the vaccine such as hepatitis B and Hib was introduced in a developed country to the time it was introduced to some of these countries. More recently, there has been a significant increase in the introduction of vaccines such as pneumococcal conjugate vaccine (39 of 47), rotavirus (34 of 47), measles-containing-vaccine second-dose vaccine (MCV2) (26 of 47) and rubella (21 of 47) after its first introduction in the global north. Gavi has made this significant achievement possible through its support to eligible countries (37 of 47). Gavi has also gone into supporting immunization systems within countries, including planning which has had the impact of improved coverage and has aimed at sustainability of the immunization programs and the gains of new vaccines introduction. In addition to Gavi support, Men-Afri-Vac has been possible to be introduced in many countries due to a successful public-private partnerships with vaccine manufacturing companies. In case of rotavirus, international not-for-profit organization and other immunization partners funded by Bill and Melinda has been key in providing technical assistance thus increasing access to and effectiveness of existing commercial rotavirus vaccines worldwide. The acceleration in the introduction of IPV and bivalent OPV is part of the Polio Eradication and Endgame Strategic Plan 2013–2018, that requires eventual cessation of all the 3 types' oral polio vaccine [9]. Therefore, the Polio Eradication Initiative has been working with countries to introduce IPV and to switch trivalent to bivalent OPV.

When we assessed the relationship of the increase in the NUVs introduction between Gavi and self-financing countries we found no significant differences in the introduction of many of the NUVs. The likely explanation is that all countries (Gavi eligible or not) supported the accelerated introduction of NUVs and assumed responsibility for their immunization programs. It could also be

that self-financing countries (10 of 47), many of which have are small and have a higher GNI per capita are able to finance the new introduction of vaccines including financing vaccines that are not universally recommended in the EPI such as mumps and influenza.

This does not suggest that in the coming years, low and middle-income countries scheduled to transition from Gavi support due to a raised GDP per capita will fund their immunization programs successfully with own money. There are other additional core issues besides financing of new vaccines after Gavi ceases support to poor countries. Although graduating countries may fully fund the purchase of vaccines, however, in the absence of good procurement practices and functional regulatory system, transitioning will be hard to achieve. The introduction of new vaccines overwhelms the vaccine supply chains; subsequently immunization coverage drops and this hinders further introduction of new vaccines. In the past, shortages have affected the supply of yellow fever, pneumococcal, rotavirus, polio and HPV vaccines due to manufacturing issues that caused delays and disruption to the routine and non-routine immunization programmes. For example, due to shortages and insufficient stockpiles, vaccines can be diverted away from the routine immunization services to outbreak responses. To meet goals of the DoV and GVAP, countries are expected to show commitment towards immunization and ensure financial sustainability of immunization programs: by raising their funds from national budgets; establishing legal framework that guarantee adequate domestic financing and procurement; and resource mobilization from development partners to meet funding gaps.

According to the Gavi annual progress report for 2015, Ghana and Sudan, for example, only funded 18% and 13% of total immunization expenditures, respectively [10]. The demonstrated budget commitment for middle-income countries like Ghana that is expected to transition from Gavi support, and Malawi that does not pay for any of their traditional vaccines raises doubts if these countries will be financially independent and ready to extend immunization and new vaccines to their targeted population after exit from Gavi support [10]. These scenarios raise serious concerns to whether low income countries will be able to mobilize their resources, ensure financial sustainability and take full responsibility of their programs in the foreseeable future.

As many countries transition out of Gavi support, there is need for collaborative partnerships between countries to work together towards purchasing affordable vaccines to maintain and further extend coverage. Collaboration is also needed between countries to explore possibilities for the development of vaccines in the region to reduce costs. With only two current vaccine manufacturers in Africa (South Africa and Senegal), investment in regional vaccine production will also greatly reduce the time to introduction of new vaccines. Most importantly it will guarantee supply security, control over production scheduling, control of costs, contribute to socio-economic development, and ensure rapid response to local epidemics and sustainability [11]. Countries receiving Gavi subsidies may be phased out soon need to plan and identify predictable local funding sources. The revolving fund, created in 1979 by PAHO for Member States is a highly impressive example that has yielded success in national immunization programs throughout the PAHO Region [12]. Some countries have established trust funds or used dedicated tax revenues, among other strategies to fund their immunization programs [4]. These initiatives are required in Africa especially now that the introduction of NUVs such as mumps, HPV, meningococcus, seasonal influenza, second dose of measles containing vaccines must be stepped up in the region.

Strengthening of the performance of the health system is essential to additional progress and sustaining the any gains made. A strong health system is a vehicle to facilitate successful introduction of NUVs and improved immunization coverage. Where exist-

ing delivery health system infrastructures are weak, the introduction of NUVs can place a hefty burden on several critical functions and this may weaken the whole health system proving to be a setback [13]. NUVs affect vaccine delivery and logistics as they become widely administered. They not only increase the number of vaccines and antigens covered by the immunization program but the increase also comes from the volume of these new vaccines which are often presented as single dose and prefilled syringes. Zaffran and colleagues estimated that for countries introducing pneumococcal conjugate vaccine and rotavirus vaccine the volume increase is 143% per dose, with a serious effect on cold chain capacity and the supply chain [14]. Furthermore, with increase in cold chain storage capacity is the corresponding increase in transport requirements. Haidari and colleagues, raises this point and laments the limited focus on cold chain capacity requirements by WHO and donors that does not consider and plan for additional transport requirements when planning for new vaccine introduction [15].

While introduction of NUVs presents challenges, it also creates extraordinary opportunities for enhancing routine immunization programs by implementing many of the required reforms. NUVs provides an entry point that pave way for scaling up and accelerating health care performance [16]. NUVs facilitate the widespread use of auto-disable syringes into immunization and the broader health systems [17]. In instances where an addition of a new vaccine does not correspond to the timing of the other doses in the schedule, the new vaccine means an additional visit to health facilities and thus provide an opportunity for additional health system contact for catch up immunization for eligible populations who previously missed vaccines. NUVs have reduced the incidence and prevalence rates of targeted diseases. For example, human rotavirus vaccine significantly reduced the incidence of severe rotavirus gastroenteritis by 30.2% among African infants in two countries during the first year of life [18]. In Rwanda, acute gastroenteritis admissions fell by 45–49% of all admissions among children below 5 years following the introduction of rotavirus vaccine in 2012 [19]. Ghana's introduction of pneumococcal and rotavirus vaccines at the same time in 2012 although costly (US\$ 3.2 million per year) was expected to prevent about 20% of the country's under-five child mortality due to pneumonia and diarrhea [20]. A study in Kenya assessing the impact of Hib vaccine introduced in 2001, reduced the incidence of the disease from 62.6 per 100000 in 2001 to 4.5 per 100000 in 2004–14 [21].

This study has limitations. The JRF is self-reported data completed by country immunization authorities of WHO Member States and we were unable to validate beyond the data published by WHO. It would be beneficial if the JRF data in future is verified by third parties in order to increase confidence in the data reporting system. We deliberately excluded other WHO regions in the analysis of the JRF data thus we did not compare how WHO African region performed at the global level and could not draw lessons on introduction of NUVs from other WHO regions. This work will be explored separately.

5. Conclusion

While notable progress has been made by many countries in the WHO African region and this is commendable, NUVs introduction remains largely dependent on Gavi support. Self-financing countries such as South Africa, Seychelles and others are making commendable progress in maintaining the introduction of NUVs. Their financing models should be shared with other countries for them to learn and plan ahead. The largest barrier to introduction of NUVs remains the cost of vaccines and current dependence of most African countries on Gavi support. Without funding for NUVs, countries would not be able to pay for the purchase of NUVs. Thus

long-term financing of NUVs is much needed and countries must begin to devote to planning and preparations related to funding mechanism and sustainability. Countries migrating from Gavi to self-financing need concerted efforts to ensure a step-wise progressive transfer of ownership of the whole immunization programs to local governments to ensure that systems and finances are not just committed on paper. As many countries transition out of Gavi funding in 2020, it is important for these countries to maintain their existing immunization programs and continue to introduce new vaccines driven by the disease burden of their populations. There is need for political and administrative endorsement of immunization policies and support systems that ensures commitment and sustainability. There is also a need for countries in the region to look into pooled funding mechanism and local development of vaccines that will help ensure sustainable immunization programs for future generations in Africa.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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Author contributions

Analyzed the data: EZS. Interpretation of the data: EZS, ABW, NN, SM, CSW. Wrote the first draft of the manuscript: EZS. Contributed to writing of the manuscript: EZS, ABW, NN, SM, CSW. ICMJE criteria for authorship read and met: EZS, ABW, SM, NN, CSW. Agree with manuscript results and conclusions; EZS, ABW, SM, NN, CSW.

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