BRIEF REPORT

Genetic analysis of measles virus nucleocapsid gene identifies measles virus isolate of close similarity to Clade A viruses from Nigeria [version 1; peer review: 1 approved with reservations, 1 not approved]

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Abstract

Previous studies on the molecular epidemiology of measles virus in Nigeria shows that genotype B3 clusters 1 and 2 are the circulating measles genotype. We report the isolation of measles virus strain of close similarity to reference genotype A measles virus strain from Ibadan, Nigeria. Molecular characterization of a measles virus isolate from a child presenting with fever and rash in a hospital in Ibadan, Nigeria, was done by measles virus isolation in Vero slam cell line, RT-PCR and direct sequencing of the COOH terminal of the nucleoprotein gene of the measles virus isolate. Phylogenetic analysis of the sequence shows that isolate MViIbadan, NIE/11.01 clustered with the reference strains of Clade A. Our current report shows evidence of another circulating MV genotype different from the previously reported genotype B3 in Nigeria. We advocate for expanded national molecular surveillance of measles virus as this will aid in achieving the country's goal of control of the disease.

Keywords

Measles virus, Phylogeny, Clade A, Nigeria

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Approval Status

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Any reports and responses or comments on the article can be found at the end of the article.

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Introduction

Measles is an acute viral illness characterised by a prodromal illness of fever, coryza, cough, conjunctivitis and presence of Koplik spots followed by the appearance of a generalised maculopapular rash. The illness is either mild or severe depending on the immune and nutritional status of the infected person. It is caused by measles virus (MV), which is a negative sense single stranded RNA virus of the family Paramyxoviridae, genus Morbillivirus¹. The virus is relatively antigenically stable and monotypic but has genetic variation in the hemagglutinin (H) and nucleoprotein (N) genes². Measles is one of the leading causes of death among young children despite the availability of a safe and cost effective vaccine for over 40 years³. It accounted for over 145700 deaths in 2013 globally, 51% of which are from World Health Organization (WHO) defined African region⁴. Nigeria still ranks high among countries reporting measles infection yearly, with over 7000 suspected and over 3000 confirmed cases reported in 2014⁴.

The 450 nucleotides that code for the COOH-terminus of the N protein is the single most variable part of the measles genome and analysis of this part of MV isolates or clinical specimens has helped to classify measles into eight clades (Clades A-H) and 23 genotypes⁵. Different measles genotypes and strains circulate in specific geographical regions^{5,6}. Measles virus clades B3, D4, D5, D8 and H1 were isolated in the WHO Americas region between 2007 and 2009, genotypes D6 and D9, in addition to those found in the Americas, are also found in the European region, and genotype B3 is the major circulating strain in the Africa region in this period⁶.

Previous reports on circulating measles strains from Nigeria have indicated the circulation of only genotypes B3 cluster 1 and 2. However, due to the absence of integrated molecular surveillance in measles' elimination programs, there has been only a few sequence data of Nigerian measles isolates, especially in recent times^{7,8}. Molecular surveillance is essential in order to observe the changes in viral genotypes over time in a particular region. It is also an important tool in assessing the effectiveness of vaccination programs⁹. This study reports the molecular characterization of a previously unreported genotype of measles virus from Nigeria.

Methods

Sample obtainment

As part of the standard care routine, a nasopharyngeal swab was collected from a 3-year-old child of suspected measles infection presenting with fever, maculopapular rash, cough and conjunctivitis at the Oni Memorial Children's Hospital in Ibadan, Oyo State, Nigeria, in November 2010. The sample was transported to the lab in virus transport medium under reverse cold chain. The swab was inoculated into tissue culture flask of VeroSLAM cell line and observed for cytopathic signs for seven days.

A nasopharyngeal swab was used as the sample type because it is the [recommended site](#) by the United States Centers for Disease Control and Prevention. In addition, measles virus is shed up to 10 day after the onset of rash and after the resolution of viremia increasing the chances of viral nucleic acid detection up to the 14 day after the onset of rash.

RNA extraction and RT-PCR

Viral RNA was extracted from the throat swab collected and supernatant from the cell culture using QIAamp® Viral RNA kit by QIAGEN Valencia USA, according to the manufacturer's instruction.

Extracted RNA was synthesised to cDNA by reverse transcription using a commercial kit (SCRIPT DNA synthesis kit by Jena Bioscience® GmbH, Germany). Nested PCR was performed using two sets of primers: First round, fwd MN5 5-GCCATGG GAGTAGGATGGAAC-3; rev MN6 5-CTGGCGGGCTGTGTGT GGACCTG-3 and nested inner primers Nfla 5-CGGGCAAGA GATGGTAAGGAGGTCAG-3, Nr7a 5-AGGGTAGGCGGA TGTGTTCTTCTGG-3, as previously described by Kremer *et al.*⁸ using Applied Biosystems GeneAmp PCR System 9700 thermal cycler. Cycling conditions for both first and second reactions is as follows: 35 cycles of 94°C for 30 seconds, 55°C for 1 minute, 72°C for 1 min preceded by 94°C for 5 minutes and a final elongation at 72°C for 5 minutes⁸.

Sequencing

Purified amplicons were sequenced at Jena Bioscience Laboratory (Germany) by Sanger sequencing method using the second round primer. Sequence data obtained was edited and assembled with [Bioedit software](#) version 7.0.5, sequence similarity was determined by Basic Local Alignment (BLAST). The query sequence was aligned with reference sequences downloaded from GenBank with the help of [Measles Nucleotide Surveillance](#) (MeaNS) database. [Table 1](#) shows the names and GeneBank accession numbers of the reference sequences used for the analysis. The basis of sequence selection was predicated on selection of characterized isolates reported in publications from WHO European region laboratory^{7,8} using CLUSTALW software. Phylogenetic trees were constructed in [Mega version](#) 6.06 software using Maximum likelihood and Neighbor joining methods with p distance model and 1000 bootstrap replicates.

Results and discussion

Both the swab and tissue culture sample showed the expected 560 base pairs after nested RT-PCR. Phylogenetic analysis of the measles sample MViIbadan/NIE/11.10 sequence (cession no [LN876569.1](#)) gave a distinct BLAST search result ([Supplementary File 1](#)). From the search it was observed that the sequence with ascension no [JF727650.1](#) Leningrad is the most closely related virus sequence with our Ibadan strain. The phylogenetic tree generated revealed that the Ibadan prototype Clade A strain co-clustered with sequences close to the vaccine Edmonston Zagreb stain with 98% bootstrap value ([Figure 1](#)), suggesting the possibility of common ancestral origin. Molecular Epidemiology of Measles virus in Nigeria classifies isolates into two distinct groups within genotype B3, B3 cluster 1 and B3 cluster 2^{7,8}. The B3 genotype was assigned after exhaustive genetic analysis of MV strains from Lagos and Ibadan in 1999⁸.

Our current study describes isolation of a different strain of measles from the previously described genotypes (MViIbadan/NIE/11.10). This virus was recovered from the throat swab of a 3 year old child with no documented history of vaccination at Oni Memorial Children Hospital in Ibadan Nigeria in November 2010.

Table 1. Names and GeneBank accession numbers reference Measles virus isolates used in phylogenetic analysis of Nigerian Measles virus Clade A isolate.

S/No	Name	Clade	Accession number
1	Measles virus Leningrad-16	A	JF727649.1
2	Measles Mvs/Fukuoka.JP/26-09/69	A	AB564297.1
3	MVi/UP.IND/45.09/2	A	HQ141409.1
4	Measles virus Edmonston (AIK-C)	A	AF266286.1
5	Measles virus Schwarz/FF-8/Vacc	A	AB591381.1
6	MVi/Ibadan.NIE/8.98/11	B3	AJ232196.1
7	MVi/Ibadan.NIE/9.98/1	B3	AJ232208.1
8	MVi/Ibadan.NIE/8.98/9	B3	AJ232209.1
9	Measles virus Y-14	B1	U01998.1
10	Measles virus R-96	B2	U01994.1
11	Measles virus (clone_NY-94N)	B3	L46753.1
12	MV (strain WTF) C2	C2	X84872.1
13	MV Maryland USA-77	C2	M89921.1
14	MV Joburg-SOA-88	D2	U64582.1
15	Measles virus Chicago-1	D3	U01977.1
16	Measles virus Canada strain	D4	U01976.1
17	MVi/Bangkok.THA/12.93	D5	AF079555.1
18	MV (clone_NJ-1-94N)	D6	L46750.1
19	MVi/Illinois.USA/50.99	D7	AY037020.1
20	MVi/Vic.AU/16.85_D7	D7	AF243450.1
21	Measles virus UK140/94	D8	AF280803.1
22	MVi/Vic.AU/12.99	D9	AF481485.1
23	MVi/Kampala.UGA/51.00.1	D10	AY923185.1
24	MVi/Ibadan.NIE/97	B3	AJ232203.1
25	MV (strain_Brx)	E	X84879.1
26	MV (strain_SMa94)	F	X84865.1
27	MVi/Amsterdam.NET/49.97	G	AF171232.1
28	MVi/Gresik.INO/18.02	G	AY184217.1
29	MV China93-7	H1	AF045212.1
30	MV China94-1	H2	AF045217.1
31	H-RSV bj8969_NP	RSV	DQ780565.1

Measles virus of genotype A has previously been detected in acute cases of measles in South and North America, China, Japan, Eastern Europe, Finland and the UK and South Africa over the last 40 years¹⁰, but there had not been any report of Clade A virus in West Africa. The virus isolated in this study is from a child who had never received any form of measles vaccination and there was no activities involving measles virus vaccine in the laboratory as at the time of virus isolation. Molecular epidemiologic information has revealed circulation of several measles genotype in Africa in recent times. Clade B

viruses is reported to be endemic in central and western parts of sub-Saharan Africa while genotypes D2 and D4 has been continually detected southern and eastern parts of Africa and genotype C2 in northern Africa¹¹⁻¹⁴ but no virus of Clade A has been reported in Africa recently.

In this study, although we did not independently investigate the immediate or remote factors that could have led to the introduction/emergence of this virus genotype in Nigeria, we postulate that the virus could have been probably imported from another

Figure 1. Phylogenetic tree of the partial nucleoprotein gene sequence of the measles virus. The study isolate is shown in bold red font; clinical strains from Nigeria are shown in blue font. Clades are indicated beside the black horizontal lines. The GenBank accession numbers are indicated first in the sequence labels, bootstrap values are indicated if $\geq 50\%$, phylogenetic tree was constructed using the Neighbor joining algorithm in MEGA 6.0 with 1,000 bootstrap replicates.

continent or could have been an undetected circulating strain. This is because active molecular surveillance is not in place in Nigeria, and the last report of MV molecular epidemiology was in 2005⁸. This leaves a huge gap in research information and multiple introductions of diverse measles genotypes might have taken place undetected over time. We therefore advocate for the establishment of an institution of a national surveillance program, and the inclusion of molecular epidemiology alongside national and global authorities, such as the WHO and Federal Ministry of Health Nigeria, to help achieve measles elimination goals in the country and globally.

Data availability

The sequence of the identified isolate has been deposited in GenBank under the accession number **LN876569.1**.

Ethical statement

Ethical approval was obtained from Oyo State, Hospital Management Board (Ibadan, Nigeria), approval number OY/HMB/REC-140010, to conduct this study. The parent of the infected child reported in this study also provided written informed consent for sequencing of the sample and use in research before the sample was collected.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were received in supporting this work.

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Supplementary material

Supplementary File 1: Blast search result for MViIbadan/NIE/11.10 Genbank no [LN876569.1](#).

[Click here to access the data.](#)

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In the study entitled “**Genetic analysis of measles virus nucleocapsid gene identifies measles virus isolate of close similarity to Clade A viruses from Nigeria**”, Faneye et al. analyze sequence data of the partial nucleocapsid gene of measles virus isolates recovered from a patient presenting with fever and rash in a hospital in Ibadan, Nigeria. The authors isolated measles virus and found evidence of a hitherto unreported measles virus, genotype A, in a child without previous vaccination history.

Though the paper is clearly presented and provides seems to provide additional information on the circulating genotype of measles virus in Nigeria, the authors need to cite current literature to update readers on the molecular epidemiology of genotype A.

Measles viruses of genotype A are mostly vaccine-like, and according to the authors the Nigeria isolate clustered with the vaccine Edmoston-Zagreb. Since measles vaccination campaign in Nigeria is widespread, I will suggest that the authors use a real-time assay by Roy et al., 2017¹ that can be used to differentiate a measles case from the commonly used measles vaccine to substantiate their claim of the uniqueness (wild type measles virus of genotype A) of the isolates from the unvaccinated child.

Additionally, the sequence of the virus used in the study had unusual insertions of nucleotides (Ts and Gs). It is however not stated the number of replicates of sequencing that was carried out. To clear the ambiguity on the clustering of the sequence as shown in the tree, I will suggest that an additional sequencing of the isolate be performed.

In conclusion, there are typographical errors across the manuscript that will require correction by the authors.

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Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Virology (RNA viruses)

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 09 April 2018

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Kevin E. Brown

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This appears to be a paper that was written in 2010 about the suggested identification of a genotype A virus from a child in Nigeria who was said to have not been vaccinated. Even a cursory glance of the literature would have provided more up to date information on the current status of measles epidemiology globally and in Africa.

There is a standard convention for naming and characterizing measles sequences that has not been adhered to. For genotyping there is a minimum 450 nucleotide window, and yet the sequence submitted in the Gnebank file is only 445 nucleotides long.

Examination of that sequence file clearly indicates that there are problems with the sequence with a long run of ts followed by gs and then a second run of ts. This has never been seen in the coding region of measles viruses before and indicates a serious problem with the sequencing results. By only showing the similarity of sequences in the paper and in the supplementary file this problem is glossed over and is not discussed or commented on in the manuscript itself.

Finally, although not as comprehensive as some countries there has nevertheless been a substantial number of sequences submitted from Nigeria since 2010. Their suggestion that a genotype A virus is circulating in Nigerai has never been substantiated by any other studies.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

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