Review Form 1.6

Journal Name:	**Microbiology Research Journal International**
Manuscript Number:	**Ms_MRJI_83723**
Title of the Manuscript:	**Genetic Diversity and Molecular Surveillance of Antimalarial Drug Resistance of Plasmodium falciparum Among Hospitals Patients in Benue State Nigeria.**
Type of the Article	

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PART 1: Review Comments

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Compulsory REVISION comments	I strongly recommend author to incorporate all of the following comments please. It is my honor to review the manuscript entitled with" Genetic Diversity and Molecular Surveillance of Antimalarial Drug Resistance of *Plasmodium falciparum* Among Hospitals Patients in Benue State Nigeria." for Microbiology Research Journal International . General comments Authors should strictly follow Microbiology Research Journal International authors guideline for all section of the manuscript please **1. Abstract** Please use the correct format (**Aims, Methodology, Results, and Conclusion) in the abstract section** Please use full terms in abstract section. Avoid abbreviation in abstract section. There are many grammatical errors .please make your manuscript evaluated by English language expert Please follow the journal guideline to write the abstract and other section of the manuscript please **2. Introduction** "Malaria is a febrile illness caused by parasites of the genus *Plasmodium* and transmitted by female *Anopheles* mosquitoes (WHO 2017)" please use the correct citation method recommended for journal specifically .use Vancouver citation style throughout your documents "*Plasmodium falciparum* is the most virulent and prevalent malaria parasite in Nigeria, accounted for 99.7% of estimated malaria cases (WHO,2019)." Similar comment to the above .please use appropriate reference citation "*falciparum* is the most virulent and prevalent malaria parasite in Nigeria, accounted for 99.7% of estimated malaria cases (WHO,2019). Despite the enormous efforts in control and elimination strategies which include the distribution of long-lasting insecticide-treated bed nets (LLINs), indoor residual spraying (IRS), larval control , improved diagnosis using malaria rapid diagnostic tests (RDTs) and the availability of artemisinin-based combination therapy (ACT);" please support it with references "Nigeria had the greatest burden of global malaria cases (27%) and malaria deaths (23%) worldwide in 2019 (WHO, 2020)." Please correct reference citation style	

	"The genetic diversity of *P. falciparum* and its innate ability to develop resistance to antimalarial drugs are some of the major obstacles in control and elimination of malaria." Please use reference "Genotypes of the merozoite surface protein two (MSP2) have been used to assessed genetic diversity in Nigeria (Olasehinde *et al*., 2012; Oyedeji *et al.,* 2013; Oyebola *et al*., 2014). While, *P.falciparum* multidrug resistance-1 (*Pfmdr-1*) and *Pf*kelch 13 propeller (*PfK-13*) genes have been used to assess antimalarial drug resistance elsewhere (Patgiri *et al*., 2019 ; Zhao *et al* .,2021)." Please use appropriate reference citation. "). The Genetic diversity is one of the predominant features of *P. falciparum* infections (Hussain *et al.,* 2011). It survives the host's immune responses due to its diversity, which results from several factors such as recombination, chromosome rearrangements, antigenic variation and allelic polymorphism (Omalu *et al*;2019). Genetic diversity in the parasite regulates transmission dynamics, disease severity, anti-malarials drug resistance and impede the development of an effective vaccine against the malaria parasite, since antigenic diversity reduced the efficacy of acquired protective immunity to malaria (Apinjoh *et al*.,2019; Patgiri *et al*., 2019;Hussain *et al.,* 2011; Mohd-AbdRazak *et al*., 2016)." Please rephrase this paragraph "According to Federal Ministry of Health (FMoH,2014), chloroquine and sulphadoxine-pyrimethamine are no longer effective in treating malaria due to high treatment failures resulting from widespread resistance in Nigeria. Nigeria adopted Artemisinin-based combinations (ACTs) as a first-line treatment of uncomplicated *P. falciparum* in 2005. ACTs are made up of a rapid but short-acting artemisinin and a long-acting partner drug combined to reduce the emergence of resistance (Anderson *et al.,* 2011). Artemether–lumefantrine (AL) and artesunate-amodiaquine are the recommended artemisinin-based" please rewrite this section and put in smart way "Some studies that investigated the prevalence of mutations in the kelch13 gene did not observe any of the major mutations associated with artemisinin resistance from Nigeria (Oboh *et al*., ,2018; Abubakar *et al* .,2020; Adam *et al.,* 2021).The present study was conducted in continuation of efforts to monitor the diversity of *P. falciparum* and molecular evidence aimed at determining resistance to antimalarial drugs among patients attending Government hospitals in Benue State, Nigeria." Please rewrite your justification of your manuscript in smart way. **3. MATERIALS AND METHODS** Information for Study Area is too bulky .please try to minimize it Please specify your study periods Please specify your study design Please explicitly explain your sampling methods and procedure9 how did you get selected study population from selected hospitals?) CONSENT AND ETHICAL APPROVAL (have taken oral or written consent? Please specify	

	"Statistical Analysis: Simple percentage and Chi-squared test were used for data presentation. Statistical analysis was performed using Statistical Product and service solution (SPSS) software version 18 (IBM., USA).The significance level was considered at P≤0.05" please authors expected to write their methods data analysis and processing clearly Before writing table you should have to have some heading with results. Please use appropriate heading according to your journals guideline please. **4. RESULTS** "The genetic diversity of the*P. falciparum* isolates using *MSP*-2 Block 3 as a genetic marker showed that the frequency of isolates possessing 3D7 family with, 37(61.7%) was higher than the FC27 allele with 18(30.0%). Infections with both allelic types were identified in 5(8.3%) of parasite isolates (Plate 1 and Figure 1). There was a significant difference (χ^2=25.9, df=2, P< 0.001) between the frequency of FC27 and 3D7 alleles in the study area."please write in smart way please "Prevalence of the single nucleotide polymorphism (SNPs) in the *pf*mdr-1 and Kelch13 gene. Out of the 60 samples of 355 bp amplicons of 754-F/754-R primers, and MDR-5/MDR-6 primer pairs each digested with *Nsp1*, and *EcoRV* to assess the mutations at codon 86 and 1246 of PfMdr-1 gene, all codons remained uncut, yielding 100% of mutant alleles at codons 86 and 1246 of the PfMdr-1 gene. In contrast, the Alu1 restriction digest of 260 bp fragment of F0/R1 primer pair of *Pf*Kelch-13 gene for mutation assessment at codon 449 yielded 100% digestion into 186 bp + 74 bp indication wild type alleles (Plate 2 andTable 3)." Please write it in smart way Better for authors to separate sentences for tables 2 and 3 after removing those grammatical errors please. "Dl = 100 bp DNA size marker, Lanes 1 and 2, 355 bp fragments amplified with MDR 5/MDR 6 primers, and digested with *Eco RV HF* restriction enzyme remains uncut (mutant) Lanes 3and 4: 355 bp fragments amplified with 754 F/754 R primers and digested with *Nsp 1* restriction enzyme remains uncut (mutant) Lanes 5 and 6 260 bp fragment of Kelch 13 gene amplified with F0/R1 primers and digested with *Alu 1* Restriction enzyme cuts the fragment to 186 bp + 74 bp (wild type)" this section is not clear, so try to explain it well please . **5. DISCUSSION** Generally discussion is full of grammatical error and editing problems. There are many unclear sentences waiting for immediate correction please. "The results of this study revealed that the 3D7 parasite clones were more abundant,	

	compared to FC27 clones. Similar results was reported in Lafia North Central Nigeria by Oyedeji *et al*. (2013)." The authors are using two different ways of reference citation .please use the correct reference citation guideline correctly. "Also, the result corroborates with earlier reports from Jharkhard India, Tripura Northeast India, and Cameroon by Hussain *et al*.(2011); Patgiri *et al*.(2019) and Metoh *et al*. (2020).In contrast, the reports from Ogun State, south western Nigeria by Olasehinde *et al*.(2012); Lagos State by Oyebola *et al*. (2014) and Minna, Nigeria by Omalu *et al*.(2019) reported that FC27 had higher frequency than 3D7 alleles. In Sabah, East Malaysia and Bioko Island in Equatorial Guinea, it was shown that FC27 had higher frequency than 3D7 alleles in those areas (Mohd-AbdRazak *et al*., 2016; Chen *et al*., 2018). The observed differences could be due to differences in the rate of malaria transmission at these locations. It could also be due to natural selection which is more efficient when acting on the Msp-2 families." This section is full of grammatical error and editing probls .please correct all of them accordingly. "Usually, the high intensity of malaria transmission in an areas is characterized by high parasite diversity, and infected individuals usually carry multiple parasite genotypes (Omalu et al., 2019). Thus in low malaria transmission areas parasite populations may have few genetic diversity leading to most infections being monoclonal (Oyebola et al., 2014). Also, it could be as result of antimalarial treatment by the patients prior to their presentation to secondary health facilities.The Msp2 is a highly polymorphic marker that has been used for genotyping recurrent parasitaemia in anti-malarial drug trials, but one argument is that in such trials, treatment is likely to reduce the number of genotypes in an infected individual, which may compromise the suitability of the Msp2 antigenic markers as a genotyping tool for drug efficacy tracking (Orimadegun et al., 2008). The acquisition of immunity, the spread of the drug resistance, the condition of transmission, and the design of effective vaccines against P. falciparum and control strategies are some useful applications that may require the knowledge about Msp2 diversity in a given area (Hussain et al., 2011, Metoh et al., 2020). Resistance to anti-malarials is a major public health problem worldwide. Mutations in P.falciparum genes, including Pfmdr1 and Pfk13 are associated with variation in parasite sensitivity to a range of drugs (Berzosa et al.,2017; Ljolje et al.,2018). The Pfmdr1 mutation is known to modulate P.falciparum susceptibility to various antimalarial drugs by regulating the influx of the drugs into the parasite's digestive vacuole (Ferreira et al 2007). Single nucleotide polymorphisms in the Pfmdr1 gene such as the N86Y and D1246Y mutation lead to changes in the physicochemical properties of the transporter thereby altering its ability to bind and transfer the target drugs (Ibraheem et al.,2014; Patgiri et al., 2019). The present study revealed 100% mutation inPfmdr1 gene for N86Y and D1246Y, which may be a sign of resistance to partner drugs that are used in combination with artemisinin such as amodiaquine and lumefantrine. However, there was 100% wildtype alleles recorded in Pfkelch13gene at codon 449, indicating the absence of the G449A mutation for Artemisinin. The implication is	

	that while the artemisinin or its derivative in artemisinin based combination drugs used in the area may appear to lack resistance developed against it, the fact that the partner drug such as amodiaquine and lumefantrine may have resistance developed against it will expose the artemisinin or its derivative to early risk of parasite resistance since it may be acting alone. Thus there may be a need to carry out an in vivo drug sensitivity study with the artesunate/amodiaquine and artemether lumefantrine combinations in the study area to observe the current efficacies of these artemisinin drug combination therapies which are currently used for the treatment of uncomplicated malaria in the area of study. The above argument is consistent with the recent observation that six mutations in Pfkelch13 gene (E433G, F434I, F434S, I684N, I684T, and E688K) were identified in northern Nigeria, among which E433G and E688K were identified from isolates with the delayed clearance artemisinin based combination (Abubakar et al.,2020). With the current use of ACTs for P. falciparum malaria throughout the world, none of these mutations detected in Nigerian isolates has been fully validated in vivo or in vitro for resistance to ACTs. The genetic diversity of P. falciparum isolates in Benue State and the very high level of key mutations (N86Y and D1246Y) associated with antimalarial drugs resistance in the Pfmdr1 gene in this study is of great concern. This is because it could threaten the efficacy of partner drugs in the ACTs and thus expose artemisinin or artemether to be a stand-alone drug in ACTs that are being used in Nigeria. If this were to persist, it would lead to early development of resistance to ACT combinations. Thus, diligent surveillance is needed to monitor the susceptibility of artemisinin-based combination therapies in the Nigeria." Please use appropriate citation and avoid grammatical errors . **6, REFERENCES** Please use appropriate reference citation according to your journal guideline please And some references are old please to replace with new references please e.g "Ferreira, P.E., Holmgren, G., Veiga, M.I., Uhlén, P., Kaneko, A. and Gil J.P.(2007).*Pf*MDR1:mechanisms of transport modulation by functional polymorphisms. PLoS One. ;6:e23875." "Lopes, D., Rungsihirunrat,K., Nogueira,1,F., Seugorn,A.,Gil1,J.P., Rosário, V.E and Cravo,P.(2002) Molecular characterisation of drug-resistant *Plasmodium falciparum* from Thailand. *Malaria Journal* 1:12" "National Population Commission. (2009). Nigeria Demographic Health Survey, 2008. Abuja, Nigeria." "Orimadegun, A.E., Amodu, O.K., Olumese, P.E., and ,Omotade, O.O. (2008) Early home treatment of childhood fevers with ineffective antimalarials is deleterious in the outcome of severe malaria. *malaria Journal*;7:143." "Price,R.N., Cassar,C., Brockman,A., M. Duraisingh,M., Vugt,M.V.,White,N.J., F. Nosten,F. and Krishna,S.(1999).The *pfmdr1* Gene Is Associated with a Multidrug-	

	ResistantPhenotype in *Plasmodium falciparum* from the Western Border of Thailand. *Antimicrobial Agents and Chemotherapy* Pp. 2943–2949." "Snounou,G.and Singh,B.(2002).Nested PCR Analysis of *Plasmodium* parasites. *Methods in Molecular Medicine* 72: Pp 189-203" And authors are expected to write URL address for some of their references e.g "WHO (2017) Malaria Fact sheet Updated. Geneva: Switzerland WHO(2019). World malaria report.*Geneva,* Switzerland. WHO,(2020).World Malaria Report. *Geneva,* Switzerland.."	
Minor REVISION comments		
Optional/General comments		

PART 2:

	Reviewer's comment	**Author's comment** *(if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

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