Multi-Drug Resistant Tuberculosis Pattern in Kano Metropolis, Nigeria

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Abstract: Tuberculosis is one of the world's major causes of illness and death, mostly in low-income countries. Nigeria ranks 4th among the 22-high burden TB countries in the world and 2nd highest in Africa. Tuberculosis is both preventable and curable as long as its causative agent *Mycobacterium tuberculosis* (TB) is susceptible to antibiotics. Recently, an alarming spread of mono-and multi-drug resistant strains of the bacterium are seen, a situation poses a growing global health problem. This study aimed at determining the drug resistance tuberculosis pattern in Kano, Nigeria. The study comprised 339 sputum specimens collected from patients attending the Directly Observed Therapy Short Course (DOTS) of Aminu Kano Teaching Hospital and Infectious Disease Hospital, Kano, Nigeria. Detection of multi-drug-resistant strains of MTB was done using the **Geno**Type® MTBDR*plus*. Sputum specimens were divided into 2 groups: **group I** comprised 298 specimens collected from newly diagnosed TB before starting treatment and **group II** comprised 41 specimens collected from patients who were on anti-tuberculous treatment. Result showed that among group I, 9 specimens (3%) were multi-drug resistant, one/was 1NH mono-resistant and one (0.29%) was RIF mono-resistant. Among group II, 27 specimens (65.8%) were multi-drug resistant, a percentage far exceeding the WHO predictions (0-4.3%); 4 specimens (9.7%) were 1NH-mono-resistant and one (2.4%) was RIF mono-resistant. No significant association was observed between drug resistance and age groups (p=0.531) or sex (p= 0.508). This research is aimed at determining the multi-drug resistant tuberculosis pattern in Kano metropolis, using the Genotype MTB DR plus.
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1. Introduction

Mycobacterium tuberculosis is the organism responsible for tuberculosis (TB) that causes about 8.8 million cases with nearly 2 million death each year, most of them in low-income countries (IUATLD, 2006). TB is curable as long as its causative organism is susceptible to antibiotics (Hauries, 2007). Recent years have seen an alarming spread of drug resistant strains of the bacterium including the emergence of multi-drug resistant (MDR) strains and extensively drug resistant (XDR) strains (IUATLD, 2006).

Multi-drug resistant TB (MDR-TB) defined by resistance to any of the first-line of drug specifically isoniazid (INH) and rifampicin (RIF) is a growing global health problem (Chan,2008, Maxmen,2010). MDR-TB emerges as a consequence of poor adherence or anti-TB treatment (WHO, 2003; WHO, 200). Multi drug resistance tuberculosis (MDR-TB) poses a serious threat to global health because it requires treatment for a long duration, frequent hospitalisation and subsequent higher cost of treatment and results in a considerable mortality [Hauris, 2007]. According to estimates by the world Health Organisation (2007) around 10% of all new TB infections are resistant to at least one anti-TB drug.

The exact TB burden in Nigeria is not known. It has been estimated to have the 4th highest burden in the world and 2nd highest in Africa (R.F). The exact prevalence of MDR-TB is also not known, however an estimate of 1.9% among re-treatment cases has been made [Onwujekwe, 2008].

2 Material and Methods

Clinical specimens: a total of 339 TB smear positive sputum species were included in the study. Sputa were collected from patients attending the Directly Observed Therapy Short course (DOTS) of Aminu Kano Teaching Hospital and Infectious Disease Hospital, Kano Nigeria. Baseline demographic data (including: age, gender, residence address, evidence of previous anti-TB treatment) was compiled from patient's medical records.

Specimens were classified into 2 groups: group I comprised 298 specimens collected from patients who had just presented with TB but never had received any anti-tuberculous treatment while group II comprised 41 specimens collected from patients who were under anti-tuberculous treatment and were attending the clinics for follow up.

All specimens were investigated for drug resistance tuberculosis using the direct MTB-DR plus test. The study was approved by the ethics committee of the hospitals.

Specimen processing: sputum specimens were decontaminated by the conventional N-acetyl-l-cysteime NaOH (NACL-NaOH) method as described before (Garnich *et al*, 1994).

Genetic identification of mycobacterium tuberculosis complex and mutations conferring resistance to isoniazid (INH) and/ or Rifampicin (RIF) was done using the Genotype MTB plus test, following manufacturer's instructions. The whole procedure consisted of 3 steps as follows:

1- DNA extraction: 1 ml of decontaminated and concentrated sputum sample was centrifuged at 10,000xg. The supernatant was discarded and the pellet containing the organisms was resuspended in 100ml of molecular biology-grade water and Incubated at 95°Cfor 20min followed by gentle sonication for 15min. The suspension was centrifuged at 22,000xg for 5 min and supernatant was collected to be used for amplification [Lacoma *et al*, 2008]

2- A multiplex amplification: in which 35 µl of biotinylated primers-nuceotide mixture (provided by the kit), 5 µl of a buffer (containing 2.5 mM $Mgcl_2$, 1.25U taq DNA polymerase) and 5 µl of the test sample, 5 µl distilled water was combined to make a final volume of 50µl/reaction. Amplification was performed in an automated theromocycler (GeneAmp PCR system 9700). The amplification protocol consisted of 15min. of denaturing at 95°C, 10 cycles comprising 30 rec. at 95° C and 120 sec. at 58°C, additional 30 cycles comprising 20 sec. at 95°C, 40 rec. at 53°C and 40 rec. at 70°C with a final extension at 70°C for 8 min. (Hain, 2008).

3- Reverse hybridization step using MTBDR plus strips was done as previously described (Barnard *et al*, 2008).

The MTBDR strip contains 27 reaction zones including (6) controls for verification of the test procedure (conjugate, amplification, MTB complex, *rpoB*, *KatG* and *inhA* controls). For detection of rifampicin resistance: (8) *rpo*B wild type (WT) and (4) mutant *rpoB* probes for detection isoniazid resistance: (1) Kat G wild type (WT) and (2) Kat G mutant probes, (2) *inhA* wild type (WT) and (4) inhA mutant probes.

Briefly biotin labelled amplicons were hybridised to the single stranded membrane bound probes. Hybridization was performed in a shaking incubator set at 45°C for 30 min. after stringent washing, a streptavidin alkaline phosphatase washing, a streptavidin alkaline phosphate conjugate was added to the strips. Strips were dried and attached to a provided evaluation/template sheet for reading.

Reading: alkaline phosphate mediated staining reaction pattern was observed, where amplicon and probe were hybridized for each strip, the bonding pattern was interpreted in two stage process. First, determination of the presence or absence of M.TB strips positive for MTb, then susceptibilities to RIF and INF were assessed.

The test sample was considered resistant to the respective antibiotic by observing either the presence of bands in each drug resistance-related gene or absence of at least one of the wild type (WT) bands. When all wild type (WT) probes of a gene stain positive with no detectable mutations within the region examined the sample tested was considered susceptible to the respective antibiotic.

Multidrug resistance was defined by detection of resistance to both RIF and INH and mono-drug resistance was defined by resistance to either RIF or INH (WHO, 2007).

Statistical Analysis

The statistical package used for the analysis of results was Epi-Info 16.0 version. These help determine the most prevalent site, the most resistant drugs, mono-drug or multi drug resistant within the region.

3. Results

A total of 339 TB smear positive sputum specimens from 339 patients of all ages attending Directly observed therapy short course (DOTS) clinic, at Aminu Kano Teaching Hospital (AKTH) and Infectious diseases hospital (IDH), all in Kano were assayed using the GenoType MTBDRplus technique.

Out of the 339 specimens analysed,298 were from patients that has just presented with TB but have never received any anti tuberculous treatment (group I)(treatment naïve), otherwise known as diagnosis patients, while 41 specimens were from patients who were on anti tuberculous treatment (group II), otherwise known as follow-up patients. Among the 298 patients analyzed for diagnosis examination (group I), 9 (3%) were found to be resistant to both rifampicin and isoniazid, this type of resistance is referred to as multidrug-resistant TB (MDR-TB) and is caused by bacteria that are resistant to at least isoniazid and rifampicin, the most effective anti-TB drugs. MDR-TB results from either primary infection with resistant bacteria (primary resistant) or may develop in the course of a patient's treatment (acquired resistant). One specimen was found to be resistant to only rifampicin (rifampicin mono resistant) and one specimen was found to be resistant to isoniazid (isoniazid mono resistant) as shown in table (1).

Table(1) Drug resistance and examination type

Examination	Number	MDR	INH	RIF
(GroupI)	298	9 (3%)	1 0.3%)	1(0.3%)
(GroupII)	41	27(65.8)	4(9.7%)	1(2.4%)
Total	339	36	5	2

4. Discussion

Multidrug resistant Tuberculosis (MDR-TB), poses a serious threat to global health because it requires treatment for a long duration, frequent hospitalization and subsequent higher cost of treatment, and results in a considerable number of mortality (Kim *et al*, 2007). In Nigeria, the national prevalence of MDR-TB is unknown. Our results show high rates of transmitted drug-resistant TB (3%), in treatment-naive patients. Furthermore, AFB sputum smears, using the Ziehl-Neelsen stain, lack sensitivity in identifying TB cases, and some cases of *M. tuberculosis* infection could have been missed. Since resistant bacteria are more likely to be less fit than sensitive bacteria (Andersson DI. 2006, Gagneux S. 2009). Therefore our results may represent an underestimate of drug resistance. This indicates that transmission of drug-resistant TB is a more serious problem than previously anticipated *(Ramaswamy and Musser, 1998)*.

The GenoType MTBDR plus test correctly identified mutations with a high concordance rate. In recent literature, the gene-based identification of MDR-TB has gained prominence. Furthermore, the genotypic analysis of *rpoB* for RIF resistance is thought to be sufficient for evaluating the public health threat of drug-resistant TB. However, recent reports indicate that this remains controversial (Smith*, et al:* 2012).

Nigeria moved from 4th position in 2007 to 10th in 2012 among the 22 high TB burden countries in the world and from 1st to 4th highest TB burden in Africa. (WHO-2012).

The implementation Direct Observed Therapy (DOTs) strategy in Nigeria since 1993 has achieved a case detection rate of 30% and treatment success rate of 79% which is still below the global target of 70% detection and 85% cure rate respectively. (Federal Ministry of Health-2010).

Concerns about extensively drug-resistance tuberculosis (XDR-TB) should be raised, which is a form of TB caused by bacteria that are resistant to isoniazid and rifampicin (i.e. MDR-TB) as well as any fluoroquinolone and any of the second-line anti-TB injectable drugs (amikacin, kanamycin or capreomycin). These forms of TB do not respond to the standard six month treatment with first-line anti-TB drugs and can take up to two years or more to treat with drugs that are less potent, more toxic(more side effects) and much more expensive. In future, work like these suppose to go ahead to survey the MDR-TB specimens, to see what could have been the prevalence of XDR-TB.

This study also raised concern over the significant proportion of MDR-TB among diagnosis subjects (those patients who never receive antituberculous treatments) an indication that resistant organisms exist within the communities. This is because the development of drug resistance may be a tragedy not only to the patients but to others, since other people may easily get infected with the drug resistant organism (WHO, 2010). As drug resistance in *M.tuberculosis is* due to the accumulation of mutation in the genome, thus the pool of MDR strains may likely develop extensive resistance if not managed appropriately (Malambo *et al,* 2008). Although Migiliori *et al* (2007) had shown MDR/XDR-TB to be curable and treatable using fluoroqunolones, they however, highlighted that rapid selection of drug-resistance mutants to fluoroqunolones is a well known phenomenon. Furthermore, MDR patients are difficult to treat because second line drugs must be used which are less potent than the first line drugs and are not as well tolerated.

It requires treatment for a long duration, frequent hospitalization, and subsequent higher cost of treatment and it results in considerable number of mortalities(WHO,2010) There is still need to develop new diagnostics such as the MTBDR plus or even easier and simpler diagnostics, new drugs and ultimately, a vaccine with which to fight tuberculosis. Early diagnosis and aggressive treatment of MDR/XDR can yield positive results for patients and public health.

Conclusion

Drug resistance among TB patients exists in different forms. INH and RIF mono-resistance are potential MDR, and MDR are potential XDR.

MTBDR plus is a new technique capable of providing result within 2-3days as against conventional DST which has Turn Around Time (TAT) of 3weeks to one month. Early diagnosis and aggressive treatment of Drug-resistant Tuberculosis can yield positive results for patients and public health. This work's finding suggests that negligence, mismanagement, lack of proper clinical practices and control plays a major role in the emergence of MDR.

References

1. Andersson, D. I. (2006): The biological cost of mutational antibiotic resistance: any practical conclusions? Curr. Opin. Microbiol. 9:461–465.
2. Chan, E. D and Iseman, M. D. (2008): Multidrug-resistant and extensively drug-resistant tuberculosis: a review.Curr. Opin. Infect. Dis. 21:587–595.
3. Federal Ministry of Health Nigeria (2011): Technical report: 2010 National HIV Sero-

prevalance Sentinel Survey, p 1–110 Federal Ministry of Health, Abuja, Nigeria.
4. Federal Ministry of Health (2010) Department of public health guidelines for community tuberculosis care Stop TB national Tuberculosis and Leprosy control programme Nigeria 6-9.
5. Ferguson, J. S., Weis, J. J., Martin, J. L and Schlesinger, L. S. (2004): Complement protein C3 binding to Mycobacterium tuberculosis is initiated by the classical pathway in human bronchoalveolar lavage fluid. *Infect Immun.* 2004;72: 2564-2573.
6. Gagneux, S. (2009): Fitness cost of drug resistance in Mycobacterium tuberculosis. Clin. Microbiol. Infect.15. (1): 66–68.
7. Global Tuberculosis Programme/WHO (2003): Treatment of tuberculosis: guidelines for national programs, 3rd ed World Health Organization, Geneva, Switzerland.
8. Granich RM , Oh P, Lewis B, Porco TC, Flood J. Multidrug resistance among persons with tuberculosis in California, 1994-2003, JAMA. 2005; 293:2732-9.
9. Hain Lifescience (2009): GenoType MTBDRplus manual, p 23–42 *In* Molecular genetic assay for identification of resistance to rifampicin and/or isoniazid of the Mycobacterium tuberculosis complex. Hain Lifescience GmbH, Nehren, Germany.
10. Harris, A.D. (2007) Robert Koch and the discovery of the Tubercle Bacillus: the challenge of HIV and Tuberculosis 125 years later. *International Journal of Tuberculosis and Lung Disease* (IJTLD). Vol. 11, no. 11, Nov., 2007 Supplement 1.
11. Huang, W. L., Chen, H.Y., Kuo, Y. M. and Jou, R. (2009): Performance assessment of the GenoType MTBDRplus test and DNA sequencing in detection of multidrug-resistant Mycobacterium tuberculosis. J. Clin. Microbiol. 47:2520–2524.
12. IUATLD (2006): Activity Report of the International Union Against Tuberculosis and Lung Disease. Pp. 16-17.
13. Maxmen A. (2010): Fighting the monster. Nature 466:S18–S19.
14. Migliori, G. B., Hopewell, P. C., Blasi, F., Spanevello, A. and Raviglione, M. C. (2006): Improving the TB case management: the International Standards for Tuberculosis Care. Eur Respir J. 2006; 28: 1–4.
15. Ramaswamy, S. and Musser, J. M. (1998): Molecular genetic basis of antimicrobial agent resistance in Mycobacterium tuberculosis: 1998 update. Tuberc. Lung Dis. 79:3–29.
16. Lana Dinic, Patrick Akande, Emmanuel Oni Idigbe, Agatha Ani, Dan Onwujekwe, Oche Agbaji, Maxwell Akanbi., Rita, Nwosu Bukola., Adeniyi, Maureen Wahab., Chindak, Lekuk., Chioma Kunle-Ope., Nkiru, Nwokoye. And Phyllis, Kanki. (2012): Genetic Determinants of Drug-Resistant Tuberculosis among HIV-Infected Patients in Nigeria J Clin Microbiol. 50(9): 2905–2909.
17. Onwujekwe, D. (2008) Epidemiology, Diagnosis and Treatment of Tuberculosis. National Training Workshop for National and Zonal TB Reference Laboratory Staff on Culture and Drug Susceptibility Testing.
18. Smith, S. E., Kurbatova, E.V., Cavanaugh, J.S., Cegielski, J.P. (2012): Global isoniazid resistance patterns in rifampin-resistant and rifampin-susceptible tuberculosis. Int. J. Tuberc. Lung Dis. 16:203–205.
19. World Health Organization. (2007): The global MDR-TB and XDR-TB response plan. World Health Organization, Geneva, Switzerland.
20. World Health Organization (2011): Nigeria: tuberculosis profile 2010. World Health Organization, Geneva, Switzerland:
21. World Health Organization (2012) Global tuberculosis report 2012, WHO/HTM/TB/2010.6.
22. Granich RM , Oh P, Lewis B, Porco TC, Flood J. Multidrug resistance among persons with tuberculosis in California, 1994-2003, JAMA. 2005; 293:2732-9.

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