The prevalence of tuberculosis and leprosy in primary health care levels in rivers state, Nigeria

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ABSTRACT

The prevalence of tuberculosis and leprosy in primary health care level was investigated in the 23 Local Government Areas of Rivers State, Nigeria. Data was collected through TB and Leprosy summary from each LGA to Ministry of Health. The case findings for tuberculosis in all the LGA's increases from 427, 1166, 2997, 3685 to 4519 and leprosy from 24, 112, 172, 189 to 229 from 2001- 2005 respectively. The prevalence was higher in the upland than the riverine areas of the state. Port Harcourt recorded the highest number of cases for TB and Tai for leprosy in the upland LGA's; whereas Ogba/Egema/Ndoni recorded the highest number of cases for both TB and Leprosy in the riverine areas of the state. The case holdings of TB increases by 92.78% and leprosy by 93.05% from 2001-2005. Treatment outcome decline from a high of 17.05% in 2001 to 15.5% in 2005. The treatment outcome of leprosy also decline from 29.0% to 18.8% to account for the increase in case holding of 93.0% during the corresponding period. There was however no significant decrease in death rate for TB and Leprosy during the period. The cure rate of TB and Leprosy diminishes from 17.05-15.53% and 29.03-18.83% respectively from 2001-2005. The number of death increases by 91.57% and 75.0% respectively for TB and Leprosy in the same period. Laboratory screening of patients increases tremendously from 1607 to 27436 patients with an increase of 63 positive cases in 2001 to 3807 cases in 2005. The prevalence of TB and Leprosy as observed showed the ineffectiveness of the DOTS approach as a management strategy.

Key words: Prevalence, Directly Observed Treatment Short Course, Tuberculosis, Leprosy, Nigeria.

INTRODUCTION

Tuberculosis possibly remain the single largest infectious cause of death in the world, with almost three million deaths annually^{1, 2}. Similarly, leprosy remains also a major chronic infectious scourge with world-wide public health implication. Tuberculosis is expected to claim 15 million lives in the next decade³. Both diseases burden on public health are exacerbated by HIV/AIDS pandemic⁴. The economically productive age group is the most vulnerable and worst affected⁵. Poor adherence to self administration of treatment is a common behavioral problem⁶, among patients suffering from chronic diseases including leprosy^{7,8,9} and TB⁶. TB can be cured in all patients by the universal acclaimed strategy- Directly observed Treatment, Short course (DOTS). DOTS is management package that guarantee effective diagnosis and treatment of infectious cases. The DOTS strategy has been described as the most important public health breakthrough in TB management considering the lives that will be saved¹⁰.

Eight out of ten patients suffering from TB treated are cured by the DOTS approach and this account for its adoption by over 110 countries of the world³. An evaluation of the effectiveness of

DOTS in different countries TB control program by the International Union Against Tuberculosis and Lung Diseases (IUATLD) observed that Malawi, Mozambique, Nicaragua, Tanzania, South Africa and Botswana achieved a cure rate of 80% mainly by Institutional Based Directly Observe Therapy (IBDOT)¹¹. The five fundamental principles of the WHO recommended DOTS strategy are: effective political and administrative will; aggressive case findings primarily by microscopic examination of sputum of patients presented to the health facilities; short course chemotherapy given under direct observation; systematic monitoring and accountability for every patient diagnosed; and adequate drug supply¹².

DOTS strategy was adopted by the Rivers State Ministry of Health, TB and Leprosy programme, in the management of these two scourges following the support of the German Leprosy and Tuberculosis Relief Agency. This study was executed to carry out a retrospective investigation of the effectiveness of DOTS strategy in the primary health care levels in Rivers State from 2001-2005. Before this intervention, there was mere attendance at the drug collection centre or clinic to received drugs by TB and Leprosy patients. This is not known to be a reliable indicator of regular drug administration.13 Adherence to self administration of Multibacillary Drug Therapy (MDT) among leprosy patients who received the entire supply of MDT at the time of diagnosis remains poor^{3,14,15}.

Also unsupervised outpatients TB treatment has often associated with poor cure rate, despite the existence of proven drug regimens¹⁶. Poor adherence had been identified as a serious obstacle to completion of treatment among individual patients, as a major barrier to the control and elimination of TB at the primary health care level, and also as one of the important causes of the emergence and rapid increase of drug resistant, particularly multi-drug resistant strains of *mycobacterium tuberculosis*^{17,15}. Failure in clinical and bacterial quiescence in many leprosy patients after prolong period of dapsone therapy is attributed to poor adherence^{13,9}.

At the primary health care level, in Rivers states of Nigeria, the counterpart funding of TB and

Leprosy from the Local Government area is appalling and persistent. Lack of appropriate incentives for the TB and Leprosy Officers often mitigate the achievement of the DOTS strategy and aggressive tracing of defaulters' cases and contacts. Many cases of non- adherence and inappropriate follow up worsen the burden of the diseases. The intervention of the German Government Tuberculosis and Leprosy Relief Association through supply of free medication has been ongoing over a decade in Rivers State of Nigeria. This study seeks to assess the impact of this intervention on prevalence of diseases burden at the primary heath care levels in Rivers State of Nigeria.

Aims and objective of study

- To carry out a retrospective evaluation of the prevalence of Tuberculosis and leprosy situation in Rivers State from 2001-2005.
- To compare the tuberculosis and leprosy situation in upland and riverine LGA's areas of Rivers State.
- To assess the effectiveness of DOTS intervention strategy and analyzing the health indices: incidence, morbidity and mortality at the local authority level and state wide level;
- Discuss the factors leading to the prevalence of the diseases in Rivers State.

MATERIAL AND METHODS

Study areas

The study was executed in Rivers State in the south-south geopolitical zone of Nigeria. The 23 Local Government Areas of the state was covered in the study. The population of Rivers states is estimated to be 5,185,400.18 In upland rural areas the occupation is predominantly agrarian subsistence farming and in the riverine community it is subsistence fishing with abysmal family income. Though oil exploration and exploitation remain common indices between the upland and riverine local government areas. Health services are offered in primary and secondary health facilities in all the Local Government Areas in addition to two tertiary health facilities in Port Harcourt, the capital of Rivers State. Curative health services are offered in 288 health centers and only 135 Health centers have Tuberculosis and Leprosy (TBL) units in the 23 LGA's in Rivers State. 18 out of 23 LGA's have laboratory diagnostic facilities based on microscopy of Acid Fast Bacilli (AFB). The anti-TB drugs: rifampicine, isoniacid, pyrazinnamid and ethambutol in fixed drug combinations are supplied in the health facilities. Specific health workers responsible for case findings, treatment, and tracing of defaulters and contacts as his or her daily work schedule are employed as the LGA's Tuberculosis and Leprosy Control Officer (LTBLO) under the co-ordination of a State TBLCO from the Ministry of health at the State level. The latter has the responsibility of management and reporting of all TB and leprosy activities. Referral services are initiated in pharmacies, NGO's and Private Clinics to health facilities with curative and diagnostic services.

Study design

The study design was focused on extraction data submitted on monthly basis from the 23 LGA's Tuberculosis and Leprosy Control Officers (LTBLO) to State TBLCO at the Ministry of Health, Port Harcourt, Rivers State, Nigeria. Personal challenges experienced at the local government levels by the supervisors in their reports were incorporated in this study.

Data Collection

The information collated from the report of the TB and Leprosy from each LGA includes:

- 1. Cases on register (COR)
- 2. Cases removed from register (CRFR) either due to death or were cured.

LGA	Population	Ca	ise findin	gstuberc	ulosis	Ca	ise findir	ngslepros	sy
	2005 census	2001	2002	2003	2004	2001	2002	2003	2004
Ahoada East	166747	41	83	204	222	2	20	21	22
Ahoada West	249425	34	43	82	87	4	4	5	5
Abua /Odua	282988	37	58	127	139	6	6	14	15
Emoha	201901	21	84	166	230	0	0	1	1
Ikwerre	189726	15	70	104	132	0	2	2	3
Etche	249454	7	43	71	79	1	1	4	5
Omuma	100366	7	22	43	49	1	2	5	6
Obio/Akpor	464789	30	85	277	345	0	0	2	3
Port-Harcourt	541115	115	314	826	1087	3	5	11	15
Eleme	190884	16	50	145	173	0	0	2	3
Ovigbo	122687	5	16	48	64	0	0	0	0
Khana	294217	20	65	271	313	0	6	13	17
Gokana	228828	15	37	108	129	1	8	16	17
Tai	117797	5	74	108	124	6	51	62	62
Akuku-Toru	156006	6	10	25	35	0	0	1	1
Asari-Toru	220100	5	12	27	29	0	0	0	0
Andoni	211009	2	6	44	49	0	0	0	1
Bonny	215358	7	10	38	47	0	0	1	1
Degema	249773	6	8	36	39	0	3	3	3
Ogba/Egbema /Ndoni	284010	24	61	156	197	0	4	8	8
Ogu/Bolo	74683	4	7	29	39	0	0	1	1
Opobo/Nkoro	151511	1	4	12	16	0	0	0	0
Okirika	222026	4	4	50	61	0	0	0	0
TOTAL	5,185,400	427	1,166	2997	3685	24	112	172	189

Table 1: Tuberculosis and leprosy situation in rivers state Iga's, nigeria (2001-2004)

LGA	Population		Case f	indingstu	berculosis			Case 1	indingsle	orosy	
UPLANDLGA'S	2005 census	2001	2002	2003	2004	TOTAL	2001	2002	2003	2004	TOTAL
1.Ahoada East	166747	41	83	204	222	550	2	20	21	22	65
2.Ahoada West	249425	34	43	82	87	246	4	4	5	5	18
3.Abua /Odua	282988	37	58	127	139	361	9	9	14	15	41
4.Emoha	201901	21	84	166	230	501	0	0	-	-	0
5.Ikwerre	189726	15	70	104	132	321	0	0	0	ю	7
6.Etche	249454	7	43	71	79	200	-	÷	4	5	11
7.Omuma	100366	7	22	43	49	121	-	0	5	9	14
8.Obio/Akpor	464789	30	85	277	345	637	0	0	0	ю	5
9.Port-Harcourt	541115	115	314	826	1087	2342	с	5	11	15	34
10.Eleme	190884	16	50	145	173	384	0	0	0	ю	5
11.Oyigbo	122687	5	16	48	64	133	0	0	0	0	0
12.Khana	294217	20	65	271	313	699	0	9	13	17	26
13.Gokana	228828	15	37	108	129	289	-	œ	16	17	42
14. Tai	117797	5	74	108	124	311	9	51	62	62	181
TOTAL	3400924	368	1044	2580	3173	7165	24	105	158	174	451
RIVERINELGA'S											
15.Akuku-Toru	156006	9	10	25	35	76	0	0	-	-	0
16.Asari-Toru	220100	5	12	27	29	73	0	0	0	0	0
17. Andoni	211009	2	9	44	49	101	0	0	0	-	-
18. Bonny	215358	7	10	38	47	102	0	0	-	-	0
19. Degema	249773	9	8	36	39	89	0	ო	с	с	6
20. Ogba/Egbema/Ndoni	284010	24	61	156	197	438	0	4	8	8	20
21. Ogo/Bolo	74683	4	7	29	39	79	0	0	-	-	0
22. Opobo/Nkoro	151511	-	4	12	16	33	0	0	0	0	0
23. Okirika	222026	4	4	50	61	119	0	0	0	0	0
TOTAL	1784476	55	122	417	512	1106	0	7	14	15	36

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Table 2: Tuberculosis and Leprosy Situation between Upland and Riverine LGA's, Rivers State, Nigeria

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- 4. Cure rate (C-R)
- 5. Death/ Died (D)
- 6. Total positive (TP)
- 7. Total Negative (TN)
- 8. Total Examined (TE).

RESULTS

Case findings LGA by LGA'S

The number of new cases (case findings) of TB and leprosy in all the LGA increases

progressively from 2001-2004.The number of new cases of TB increased in Ahoada East LGA's from 41-222 (81.53%), Ahoada West LGA's from 34-87 (60.92%), Abua-Odua LGA's from 37-139 (73.38%), Emuoha LGA from 21-230 (90.87%), Ikwerre LGA from 15-132 (88.64%), Etche LGA from 7-79 (91.14%), Omuma LGA from 7-49 (85.71%), Obio/ Akpor LGA from 30-345 (91.30%), Port Harcourt LGA from 115-1087 (89.42%), Eleme LGA from 16-173 (90.75%), Oyigbo LGA from 5-64 (92.19%), Khana LGA's from 20-313 (93.61%), Gokana LGA

	Lepro	sy			Tuberculo	sis
Year	COR	C – R	CRFR	COR	C - R	CRFR
2001	62	1	8	1120	348	155
2002	142	4	3	3337	593	444
2003	451	9	6	9779	1787	1331
2004	564	12	8	12175	2297	1661
2005	892	19	44	15503	2938	1951

Table 3: Case holdings of TB and Leprosy 2001 – 2005

COR, Cases on register; C-R, Cure rate; CRFR, Cases removed from register

Table 4: Treatment Outcome 2001-200

	Lepro	sy			СВ	
Year	Cure	DE	%CR	CUR	DE	%CR
2001	18	2	29.03	191	15	17.05
2002	60	2	42.25	565	36	16.93
2003	105	7	23.82	1438	111	14.70
2004	130	8	23.05	1892	131	15.54
2005	168	8	18.83	2408	178	15.53

CUR, Cure; DE, Death; CR, Cure Rate.

Table 5: Laboratory diagnosis for tuberculosis 2001-2005

Year	Total Examined	Total Positive	Total Negative
2001	1607	6	153
2002	14,291	8	173
2003	17,029	20	189
2004	22,597	26	189
2005	27,436	38	324

from 15-129 (88.37%), Tae LGA's from 5-124 (95.97%), Akuku Toru LGA's from 6-35 (82.86%), Asari Toru LGA's from 5-29 (82.76%), Andoni LGA's from 2-49 (97.92%), Bonny LGA from 7-47 (85.11%), Degema LGA from 6-39(84.62%), Ogba-Ebema LGA's from 24-197 (87.82%), Ogu- Bolo LGA's from 4-39 (89.74%), Opubo/ Nkoro LGA's from 1-16 (93.75%) and Okirika LGA's 4-61 (93.44%) from 2001-2004.

The number of leprosy increased in Ahoada East LGA's from 2-22 (90.91%), Ahoada

West LGA's from 4 -5 (20.0%), Abua-Odua LGA's from 6 -15 (60.0%), Emuoha LGA from 0 - 1 (100%), Ikwerre LGA from 0 -3 (100.0%), Etche LGA from 1 - 5 (80.0 %), Omuma LGA from 1 - 6 (83.3 %), Obio/Akpor LGA from 0 - 3 (100.0%), Port Harcourt LGA from 3 -15 (80.0%), Eleme LGA from 0-3 (100.0%), Oyigbo LGA 0 (0.0%), Khana LGA's from 0 - 17 (100.0%), Gokana LGA from 1 -17 (94.1%), Tae LGA's from 6-62 (90.3%), Akuku Toru LGA's from 0 - 1 (100.0%), Asari Toru LGA's from 0 - 0 (0.0%), Andoni LGA's from 0 - 1 (100.0%), Bonny LGA from 0 - 1(100.0%), Degema LGA from 0 - 3 (100.0%), Ogba-Ebema LGA's from 0 -8 (100.0%), Ogu Bolo LGA's 0 (0.0%), Opubo/ Nkoro LGA's 0 (0.0%) and Okirika LGA's 0 (0.0%) from 2001-2004 (Table 1).

Case findings between upland and riverine LGA's

The number of new cases of TB in the upland LG Areas increased from 368, 1044, 2580, 3173 and 4519; Leprosy new cases increased from 24, 112, 172, 189 and 229 from 2001, 2002, 2003, 2004 and 2005 respectively representing an increase of 88.40% for TB and 89.52 % for Leprosy. The number of new cases of TB in the riverine LGA's increased from 55, 122, 417 and 512; Leprosy 0, 4, 8, and 8 from 2001 2002, 2003 and 2004 respectively representing an increase of 89.26% for TB and 100.0% for Leprosy (Table 2).

Case holdings

The cases on register (COR) for TB increases from 1120, 3337, 9779, 12175, and 15503; and for leprosy 62, 142, 451, 564 and 892 in 2001, 2002, 2003, 2004 and 2005 respectively. The cases removed from register (CRFR) due to either cures or deaths for TB are 155, 444, 1331, 1661 and 1951, and for leprosy 8, 31, 62, 87 and 144 in 2001, 2002, 2003, 2004 and 2005 respectively. The cure rate of TB recorded include 348, 593, 1787, 2297 and 2938; for leprosy 17, 40, 92, 124 and 190 in 2001, 2002, 2003, 2004 and 2005 respectively (Table 3).

Treatment outcome

The percentage cure for TB is 17.05%, 16.93%, 14.70%, 15.54%, and 15.53%; and for leprosy is 29.03%, 42.05%, 23.82%, 23.05% and 18.83%; the percentage death rate of TB is 1.34%,

	Case I	indinds-					Case Holc	lings	Treatme	ent out	come				
	Leprosy		TB	Leprosy				TΒ			Lep	rosy		TВ	
fears				COR	с. С	CRFR CURE	COR	CRFR	CRFR CURE	U	۵	%CR	ပ	۵	%CR
	2001	24	427	62	17-	ø	1120	246	155	18	~		191	15	64.8
01	2002	112	1166	142	40	31	3337	593	444	60	2	565	36	74.18	-
~	2003	172	2997	451	92	62	9779	1787	1331	105	7	1438	111	78.4	-
+	2004	189	3685	564	124	87	12175	2297	1661	130	œ	1892	131	75.46	2
10	2005	229	4519	892	190	144	15503	2938	1951	168	ø	2408	178	70.77	2

1.08%, 1.14%, 1.08% and 1.15%; and for Leprosy 3.23%, 1.41%, 1.53%, 1.42%, and 0.9% in 2001, 2002, 2003, 2004 and 2005 respectively (Table 4).

Laboratory diagnosis

The total number of TB cases presented for laboratory diagnosis increased from 1607, 14291, 17029, 22597, and 27436; the smear positive cases increased 63, 848, 2038, 2645, and 3807; the smear negative cases are 157, 172, 189, 189, and 324 in 2001, 2002, 2003, 2004 and 2005 respectively (Table 5). The holistic summary of the entire study is shown in Table 6.

DISCUSSION

Both Leprosy and Tuberculosis are chronic diseases caused by Mycobacterium, the species for the former is *leprae* and the latter is *tuberculosis*. Also TB and Leprosy are infectious; leprosy affects the peripheral nervous system, the skin and certain other tissues. While TB is noted to attack the lungs and certain other tissues. They most probably have a common epidemiology in a community as they depend on the proportion of susceptible persons in the population and the opportunities of contact with the bacteria. Adults are less susceptible than children who are more susceptible; 60% of those who develop the diseases are children or young adults after an incubation period of 2-7 years (usually 3 - 5 year). Vertical transmission of leprosy from mother to child may occur as a rare event¹⁹. The mode of transmission is obscure but three possible route - skin, the gastrointestinal tract and the respiratory tract are under consideration. Skin to skin contact or transmission by flies or other arthropods has been confirmed^{20,21}. Nasal secretions have been found to be vehicle²² and mosquitoes have been incriminated²³. Droplets from nasal secretions of lepromatous patients can discharge bacilli when talking, sneezing or coughing or can be absorbed by dust²⁴. Nasal mucosal damage from common cold may facilitate entry of Mycobacterium. Both are zoonosis as it affects wild animals²⁵.

Poor housing and nutrition facilitate the spread of both TB and leprosy. Overcrowding and undernourishment reduces immunity in immunoconmpromised individual. Both diseases have a wild distribution in the world and most prevalent in the tropics and subtropics; but it occurs as endemic diseases but less commonly in temperate climate²⁶. It is estimated that there is 11.5 million cases of leprosy with 5.3 million registered cases in the world. The origin and history medieval leprosy has been extensively discussed²⁷. In Rivers the prevalence of tuberculosis may be exacerbated by the ubiquitous gas flares and the release of noxious substances that increases susceptibility to lung diseases. Tuberculosis remain a challenge in most low and medium income countries of the world with over 1.8 million dead annually from TB²⁸. In Rivers State where the per capita income is less than one dollar per day, poverty, ignorance and poor adherence to drug regimen remain the major factors contributing to gross increase in TB and Leprosy case findings at all the Primary Health care Level in the 23 LGAs. Port Harcourt LGA's from this survey recorded the highest number of cases with an average of 586 new cases per year in the upland areas and Ogba/ Egbema/Ndoni LGA's recorded the least incidence with an average of 109 new cases per year in the riverine areas. Tae LGA's has an average of 45 new cases and Ogba/Egbema/Ndoni LGA's has an average of 5 new cases of leprosy per year in the upland and riverine areas respectively. Rural urban drift and the high influx of people to Port Harcourt due to expansion of oil and gas business have put devastating strains on all facilities. These have led to preponderance of poor housing, existence of slums and overcrowding conditions predispose to the spread of mycobacterium. Lack of good information, health education and communication may be responsible for the high incidence of both scourges. Furthermore HIV/AIDS have been reported to exacerbate the new incidence of tuberculosis world wide4 and Rivers state with five portal of entries is not unexpected to have favorable predispose epidemiology of communicable diseases.

The low incidence of TB and leprosy observed in the riverine areas of the state might be as a result of the difficult terrain, high cost of transportation and poor accessibility of subjects to health personnel and medical facilities resulting in low contacts tracing. These factors might not be unrelated to the low incidence of the scourge recorded in this survey.

The DOTs approach used in this study have in other studies in other countries have been found to be highly effective in reducing incidence of TB however its benefits in this study was incorrigible. The case holdings for TB increases by 92.8% and leprosy by 93.0% in all the LGA's. This high incidence of TB might be due to decrease in treatment outcome which decline from a high of 17.05% in 2001 to 15.5% in 2005. The treatment outcome of leprosy also decline from 29.0% to 18.8% to account for the increase in case holding of 93.0% during the corresponding period. There was however no significant decrease in death rate for TB and Leprosy during the period. This suggests a lot of variables such as socio-economic, cultural and environmental factors contributing to DOT's approach as an ineffective management strategy of TB in Rivers State.

As a result of limited effectiveness of DOTS in some health jurisdiction it has been advocated that evidences about effectiveness in methods and strategies other than Directly Observed Treatment (DOT) has to be explored to increase concordance for TB treatment^{29,30}.

Enhancement of the level of understanding and adaptation of care to the social support system of each person affected by care providers might be a key factor in improving concordance to treatment of this scourge. The number of both TB and Leprosy positive and negative smears during the period increases during the period thus suggesting improvement in laboratory patronage.

Lack of compliance or adherence to therapy and absenteeism among outpatients constitute the major factors responsible for the preponderance of the scourge in Rivers State. Resistance to chemotherapy used in the management of these diseases exists, for example there is resistance to dapsone^{20,22} and clofazimine¹³. This necessitates the use of fixed drug combination therapy:- dapsone, clofazimine, rifampicine, etionamide and thiacetazone for leprosy; and rifampicine, ethambutol, thiacetazone and isoniazid for tuberculosis.^{31,32,33}

The shelf life of the drugs used in the management of TB and Leprosy range from 4-5

years. But the extremes of the hot and humid climates of the tropics facilitate capsules to gummed together and enhanced faster drug degradation and loss of therapeutic value and eventual resistance to sub-therapeutic doses of chemotherapy.

Side-effects exist from drugs used in the management of this scourge. For example heamolysis have been noted in patients on dapsone who have G-6-PD (Glucose-6-phosphate dehydrogenase) deficiency, fixed drug eruptions may complicate treatment with dapsone even after drug is well tolerated; peripheral neuropathy is also common with clofazamine in addition to other gastrointestinal side effects, skin pigmentation, coloring of urine, sweat, sputum, tears, typical ichthyosis, irritation and burning discomfort and skin lesions are serious side effects associated with chemotherapy. Rifampicine-serious side effect includes hepatitis, thrombocytopenia, psychosis, osteomalacia, and hypersensitivity reaction. These side effects most often interfere with patients' drug adherence.

Prevention strategy used in endemic areas which include: case finding and prompt treatment, surveillance of families of patients, immunoprophylaxis to negative contacts, vaccination to all young children, improvement of living conditions so that members of families do not live close to contacts; advocacy, education and communication about leprosy and tuberculosis were observed in this study to be infrequently used as the counterpart funding from the LGA's were not paid regularly. Besides, paucity of remuneration to health personnel results to poor attitude to work.

CONCLUSION

The alarming prevalence in the epidemiology of tuberculosis and leprosy in Rivers state are not statistics to be ignored. Increases counterpart funding from all level of governments and committed political will to recognized TB and leprosy as scourges like HIV/Aids; proper remuneration of health staff and effective chemotherapy and concordance remains the pivot that can reduce the high prevalence and incidence of TB and leprosy observed in this study.

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