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ABSTRACT:
Drug resistant TB is increasing in Papua New Guinea. Although drug resistant data of mycobacterial isolates from pulmonary TB cases is available, there is limited information on culture confirmed drug resistant tuberculous lymphadenitis. In the framework of a pilot study evaluating the use of Xpert MTB/RIF in diagnosing tuberculous lymphadenitis compared to microscopy at the Port Moresby General Hospital, 18 fine needle aspiration biopsy samples were sent to Brisbane, Australia for culture. We report the drug susceptibility testing results of 12 isolates (12/18). The mycobaceterial yield was 66.7% (12/18) with 58.3% (7/12) of the isolates showing drug resistance to at least one drug tested. Mono-resistant rate was 25% (3/12) whereas Multi Drug Resistant TB (MDR-TB) rate was 33.3% (4/12).

Keywords: fine needle aspiration biopsy, tuberculous lymphadenitis, GeneXpert, Xpert MTB/RIF, drug susceptibility testing, Papua New Guinea
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INTRODUCTION:

The World Health Organisation (WHO) estimates that about 8 million people get infected with mycobacterium tuberculosis (MTB) each year of which nearly two million die [1]. In Papua New Guinea (PNG) the estimated incidence of tuberculosis (TB) is 532 per 100 000 per annum [2,3]. Drug resistant TB and multi-drug resistance-TB (MDR-TB) are major challenges in the fight against TB in PNG [3]. The recently completed PNG TB drug resistant survey is expected to provide accurate data on drug resistant pattern of MTB isolates in PNG [personal communication, Lavu 2015]. PNG does not have drug susceptibility testing (DST) facilities and samples that require DST are sent to the Queensland Mycobacterium Reference Laboratory (QMRL) in Brisbane, Australia. There are plans to re-establish MTB culture facilities at the PNG Central Public Health Laboratories

(CPHL) and it is expected to be operational in 2016. A review of the DST patterns of MTB isolates from the Western Province of PNG showed isolates to be resistant to at least one of the five primary drugs used for treating TB [2]. The rate of MDR-TB in that study was 26% [2].

Ley et al examined DST patterns of isolates from Goroka, Madang and Alotau that showed 10.8% of isolates were resistant to at least one drug tested where 30.4% were mono-resistant to Streptomycin, 17.4% to Isoniazid and 13.0% mono-resistant to Rifampicin [4]. In that study, isolates from Alotau had a MDR-TB rate of 4.6% [4]. These aforementioned DST patterns are from isolates obtained from sputum samples. A study in Ethiopia showed 6.7% of MTB isolates from lymph node aspirates were resistant to at least one first line anti-TB drug [1]. In that same study the MDR-TB rate was 1.3% [1]. There is limited data on the DST pattern of isolates obtained from patients with tuberculous lymphadenitis (TBLN) in PNG. We report the DST pattern of 12 MTB isolates cultured from fine needle aspiration biopsy (FNAB) aspirates at the Port Moresby General Hospital (PMGH).

METHODS:

Study design and sampling

In the framework of a prospective descriptive pilot study comparing Xpert MTB/RIF (Xpert) and microscopy in diagnosing TB lymph node at PMGH, FNAB samples requiring DST were sent to Australia for culture. Every third consecutive patient was chosen and interviewed. Study subject were recruited by purposive convenience sampling. A total of 107 study participants were recruited from the weekly FNAB clinic at PMGH. Informed written consent was obtained from all study participants. Parents and guardians of children aged 13 years and below gave written and informed consent prior to recruitment into the study. In-patients requiring FNAB for diagnostic workup were also included. Study participants were allowed to withdraw from the study at any time. Basic demographic and clinic data were obtained using a pre-tested questionnaire. Patients with breast lumps and thyroid enlargement presenting for FNAB were excluded from the study. Ethical approval for the study was obtained from the PNG National Department of Health Medical Research Advisory Committee (MRAC File Number 54-6-2).

Fine needle aspiration and laboratory testing procedure:

A 23 gauge hypodermal sterile needle was attached to a 10ml syringe and the FNAB samples were obtained without a syringe pistol. If there were multiple glands, samples were obtained from the largest gland. Samples were obtained from cervical, submandibular, axillary and inguinal lymph nodes. After cleaning the skin with 70% alcohol skin swab, while using the non-dominant hand to fix the enlarged gland and immobilising it, the dominant hand holding the syringe with attached needle was inserted into

the gland. A suction pressure of 2.0ml was applied and sample obtained by moving the needle back and forth without completely withdrawing the needle. Suction was stopped and needle withdrawn when sample was visible at the hub of the needle. Maximum of two passes were done if first obtained insufficient material. Standard smears were then made on frosted glass slides for Ziehl-Neelsen and modified Wright Giemsa staining. The remainder of the FNAB specimen in the needle hub was dispensed into a sterile container with 2ml physiological saline for Xpert testing at the CPHL. Physiological saline has been shown to be a good transport and storage medium of FNAB aspirates for Xpert analysis [4].

The WHO Xpert implementation manual and CPHL standard operating procedures were used as guide for Xpert result interpretation [6]. Specimen preparation for Xpert analysis was modified from Malbruny et al [5]. Modification was as follows:

- 1.0ml of the aspirate-saline mixture was transferred into another sterile container of 1.0ml physiological saline to make it up to 2.0ml. This was done to allow better emulsification of the mucoid or blood stained material by the Xpert sample preparation buffer and to allow enough volume to work with.
- The Xpert sample preparation buffer was added to the mixture at a ratio of 2:1.
- After votexing, the mixture was incubated at room temperature for 10 minutes. The mixture was vortexed again and incubated at room temperature for a further 5 minutes.
- 2.0ml of the mixture was dispensed into the Xpert cartridge and processed using the manufacturer's protocol (Cepheid, USA).

The remainder of the unprocessed aspirate-saline sample following Xpert testing was sent to the QMRL in Australia for DST if rifampicin resistance was detected by Xpert. The QMRL is the usual laboratory for MTB DST on samples from PNG [7]. Criteria for culture were (1) Rifampicin resistance by Xpert and (2) as a component of the quality assurance program at CPHL. Personal protection equipment was worn and standard biosafety procedures were followed at all times during the sampling and testing processes.

RESULTS:

A total of 107 patients were recruited for the study and all of them consented to participate. Thus the consent rate was 100%. However, culture was done on the sample from 18 (16.8%) patients. Mycobacterium tuberculosis complex was isolated from 12 of the 18 (66.7%) samples. The mean (± Std Dev) age of the 12 patients was 24.3 ± 8.5 years, median age 24.5 years and age range was 11.0 to 40.0 years.

Table 1: Basic demographic and clinical data of positive culture

Isolate No.	Age	Gender	Aspirate type	Positive family history	Previously treated for TB	Place of residence	MTB detected by Xpert	Rifampicin resistance detected by Xpert	Microscopy result	Resistance detected By culture
1	23	F	blood+pus	No	Yes	Rural	Yes	No	TB	Nil
2	30	F	blood+pus	No	No	Settlement	Yes	Yes	TB	Nil
3	31	F	pus	No	No	Urban	Yes	Yes	TB	RIF
4	12	M	pus	No	Yes	Settlement	Yes	Yes	TB	RIF
5	32	M	blood	No	No	Rural	Yes	Yes	TB	Nil
6	26	F	blood	No	No	Urban	Yes	Yes	TB	Nil
7	26	M	blood+pus	No	No	Urban	Yes	Yes	TB	Nil
8	11	M	blood+pus	Yes	Yes	Peri-urban village	Yes	Yes	Suppurative lymphadenitis	RIF, INH, EMB, PZA, ETH
9	23	M	pus	No	No	Urban	Yes	Yes	TB	RIF, INH, ETH
10	40	M	blood+pus	Yes	Yes	Rural	Yes	Yes	TB	RIF
11	22	M	pus	yes	Yes	Settlement	Yes	Yes	Suppurative lymphadenitis	RIF, INH, ETH
12	16	M	blood+pus	No	No	Settlement	Yes	Yes	TB	RIF, INH, ETH, O

RIF= Rifampicin, INH = Isoniazid, ETH = Ethionamide, O = Ofloxacin, PZA = Pyrazinamide, EMB = Ethambutol.

Table 2:.Basic demographic and clinical data of negative culture

Isolate No.	Age (years)	Gender	Aspirate type	Positive History	Previously treated for TB	Place of residence	MTB detected by Xpert	Rifampicin resistance detected by Xpert	Microscopy result	culture
1	1.9	M	Blood	Yes	No	Rural	No	-	Non-specific lymphadenitis	No growth
2	28	F	Blood+pus	Yes	No	Urban	Yes	Yes	TB	No growth
3	21	F	Blood+pus	Yes	No	Urban	Yes	Yes	TB	No growth
4	26	F	Blood+pus	No	Yes	Urban	Yes	No	TB	No growth
5	40	F	Blood+pus	Yes	No	Settlement	Yes	Yes	Suppurative lymphadenitis	No growth
6	52	M	Pus	Yes	Yes	Urban	Yes	No	TB	No growth

Gender distribution of the 12 patients indicated 8 (66.7%) males and 4 (33.3%) females. The mean, median and age range of the male patients were 22.8 ± 10.0 years, 22.5 years and 11.0 to 40.0 years respectively. For the female patients the corresponding values were 27.5 ± 3.7 years, 28.0 years and 23.0 to 31.0 years respectively.

Of the 12 isolates there were seven (58.3%) resistant isolates four (33.3%) of which were MDR-TB. Three patients (25%) had positive family history and 5 (41.7%) were previously treated for TB. Of the four patients with MDR-TB, only one (25.0%) had a history of previously being treated for TB. Four patients (33.3%) were from settlements, 3 (25%) from rural villages outside Port Moresby, 1 (8.3%) from a peri-urban village and 4 (33.3%) were from urban municipal residential areas in Port Moresby. Xpert detected MTB in 17 samples (94.4%) whereas microscopy was negative in 4 (22.2%). Two (50%) of the negative microscopy specimen grew MTB complex. Table 1 shows the basic demographic and clinical data of the 12 patients with positive isolates. Table 2 shows the clinical and demographic data of the six patients with culture negative samples.

DISCUSSION:

Three categories of drugs are available for use in PNG to treat TB [3]. The drugs are available for use as fixed dose combinations and are used for PTB and EPTB treatment. Category one are used for all new TB cases, category two for all re-retreatment cases and drug resistant cases are treated with category four [3]. Category one drugs includes rifampicin, isoniazid, pyrazinamide and ethambutol. Category two drugs includes all the category one drugs plus streptomycin and category four drugs are pyrazinamide, kanamycin, levofloxacin, ethionamide, cycloserine, capreomycin and para-amniosalicylic acid. MTB isolates from Western Province were shown to be resistant to all category one and two drugs except amikacin and kanamycin [2]. Isolates from Goroka, Madang and Alotau have been found to have mono-resistant rates of 8.9%, 4.6% and 6.7% respectively [4]. In our cohort of 12 isolates the mono-resistant rate was 25% (3/12). Whereas isolates from Goroka, Madang and Alotau showed mono-resistance to streptomycin and isonidazid, no isolates in our study showed resistance to these drugs. However, our results are from 12 isolates whereas Let et al analysed more than 50 isolates from each town [4].

Compared to other studies on MDR-TB in PNG our cohort had a MDR-TB rate of 33.3% (4/12).This high rate obtained in a pilot study but indicates the need for a larger sample size study at PMGH. Two of MDR-TB cases were from settlements, one from a peri-urban village and one was from a village outside Port Moresby. Two patients with MDR-TB had neither past history of being treated for TB nor positive family history. Genetic analysis of drug resistant MTB

isolates from PNG has shown identical resistance-conferring mutations within clustered isolates suggesting patient to patient transmission of drug resistant TB [7]. We did not conduct genetic analysis on the MTB isolates but the results indicate a need for molecular epidemiological studies using MTB isolates from patients at PMGH. The MDR-TB isolates showed resistant to rifampicin, ethambutol, ethionamide and pyrazinamide. This pattern is similar to other published data on TB drug resistance in PNG [2,7,4,]. Isolates in our study did not show resistance to streptomycin, capreomycin, cycloserine and para-aminosalicylic acid (PAS) which is different to other studies in PNG [2,4]. A study in Ethiopia suggests newly identified strains of MTB may have an affinity for lymph nodes causing tuberculous lymphadenitis [10]. It is not known if these new strains have a different DST pattern compared to isolates from patients with PTB.

Two positive cultures were from children of which one was a MDR-TB isolate. Obtaining positive culture in children is very challenging and using a modified sample processing procedure, MTB complex was isolated. The procedure described can be used to investigate children with TBLN that are not responding to first line TB drugs. The procedure is also useful to sample enlarged lymph nodes for Xpert analysis. Fine needle aspiration biopsy of enlarged node is cheap and easy to perform with minimal training and other health care workers can be trained to perform this procedure at the outpatient enabling sample collection for culture [11]. Availability of Xpert in 21 hospitals in PNG further offers a means of laboratory confirmation of TBLN. Readily available infrastructure and equipment for laboratory confirmation of TBLN will ensure rapid case detection and treatment of cases. Further, culture confirmation and rapid dissemination of DST results will help combat drug resistant TB in PNG.

This study had a mycobacterial culture yield of 66.7% (12/18). Culture yield of MTB from FNAB is reported to be between 42% and 83% [12]. Positive human immunodeficiency virus infection (HIV) is also associated with a higher yield from FNAB aspirates in adults [12]. We did not investigate for HIV status due ethical considerations in the study design. Factors relating to the negative culture may have included inadequate specimen (1 patient) or TB treatment for more than 2 weeks (2 patients). Although we used physiological saline for emulsifying the FNAB aspirate for culture, the yield is higher in MTB-specific transport mediums [12]. Prolonged storage of specimen prior to shipment to Australia may have also resulted in the negative cultures.

CONCLUSION:

MTB can be cultured from FNAB aspirates emulsified in physiological saline. The DST pattern of the 12 isolates from FNAB aspirates in this study suggests the possibility of a difference

in DST pattern between PTB and TBLN at PMGH. However, a larger sample size is needed to confirm these possible differences.

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