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TEACHING FILES (GRAND ROUNDS)

DISCORDANCE BETWEEN XPERT MTB/RIF ASSAY AND LINE PROBE ASSAY- HOW TO INTERPRET?

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TEACHING FILE:

A 2-year-old boy presented with a progressively enlarging painful swelling over the right cheek for 8 days. It was associated with fever. There was no facial trauma, toothache, dysphagia, neck swelling or contact with a patient suffering from tuberculosis (TB). On presentation, his weight was 11.2 kg (25th percentile as per Indian Association of Pediatrics (IAP) growth chart) and height was 88 cm (between 50-75th percentile as per IAP growth chart). There was pallor and a fluctuant, tender swelling over the right cheek with redness over the swelling measuring approximately 2.0 x 1.5 cm. Other general and systemic examination was normal. On investigation, ultrasound (USG) of the right cheek revealed a heterogeneous collection in the subcutaneous plane of approximately 4 cc volume, surrounding significant inflammation and enlarged submandibular lymph nodes suggestive of a cheek abscess. Other investigations are shown in Table 1. Incision and drainage of the right cheek abscess was done and pus culture yielded methicillin-resistant *Staphylococcus aureus* (MRSA). The patient was given oral linezolid for 5 days. *Mycobacterium tuberculosis* (MTB) was not detected on pus Xpert MTB/RIF. However, first-line line probe assay (FL-LPA) revealed KatG resistance with *Inh A and Rif* sensitivity, and second-line LPA (SL-LPA) revealed fluoroquinolone (FQ) resistance with amikacin, kanamycin and capreomycin sensitivity. On follow up, the patient was doing well, gaining weight and the surgical site was healing adequately.

Table 1: Investigations of the patient

Investigations	Patient	Reference Range
Hemoglobin (gm/dL)	12.1	11.5-15.5
White blood cell count (cells/cumm)	19820	5000-13,000
Absolute neutrophil count (cells/cumm)	11020	2000-8000
Absolute lymphocyte count (cells/cumm)	6140	1000-5000
Platelets (106 cells/cumm)	3.58	1.50-4.50

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How to interpret the discrepancy between the Xpert MTB/RIF and LPA for MTB?

Discussion:

In this scenario, there is discordance between the results of Xpert MTB/RIF and the FL-LPA and SL-LPA results. Xpert MTB/RIF is a nested real-time polymerase chain reaction (PCR) test which amplifies a segment of the rpoB gene in order to qualitatively detect MTB complex and rifampicin resistance (RR). The sequential steps of sample purification, nucleic acid amplification and target sequence detection are all automated and performed in a closed system, thus reducing the risk of cross-contamination.[1,2] It is a simple process to perform requiring minimal training and it requires biosafety level (BSL) 2 facilities. [1,3] FL-LPA is a multiplex PCR test that amplifies segments of *rpoB*, katG and *inhA* genes to qualitatively detect rifampicin, high-level isoniazid and low-level isoniazid resistance respectively.[3,4] SL-LPA is also a multiplex PCR test that amplifies segments of gyrA and rrs genes to qualitatively detect fluoroquinolone and second-line injectable drug (SLID) (amikacin, kanamycin and capreomycin) resistance respectively. LPA requires a BSL-3 lab and is more technically challenging to perform as compared to Xpert MTB/RIF.[3,5] Per protocol, the steps of sample decontamination and DNA extraction, nucleic acid amplification and hybridisation, are performed in three separate rooms and in a unidirectional fashion.[6] Despite this, the chances of contamination and subsequent false positive results on LPA, as in our case, are higher due to several reasons. Firstly, LPA can be performed on both clinical samples (direct testing) and culture isolates (indirect testing).[4] Testing on a contaminated culture isolate may lead to such a false-positive result.[3] Secondly, technical laboratory errors serve as an important source of contamination. Laboratory air and surfaces, molecular biology grade water, LPA reagents, LPA kits and lab equipment may serve as sources of DNA contamination. DNA may also be transferred directly from lab staff to the sample or indirectly from lab staff through objects. The use of personal protective equipment (PPE), while protective against contamination, if used incorrectly, may also serve as a vector. Lastly, such discrepancies may also rise from the mix-up of samples.[2] Additionally, LPA has a much higher limit of detection as compared to Xpert MTB/RIF, hence scenarios such as ours where Xpert MTB/RIF failed to detect MTB but LPA was able to detect it, can be attributed to contamination.[3]

Another possibility in this case is that Xpert MTB/RIF is falsely negative. Aricha et al., [7] compared Xpert MTB/RIF and LPA with conventional culture respectively and found that LPA had a higher sensitivity and negative predictive value than Xpert MTB/RIF when it came to detecting MTB. Rufai et al.,[8] conducted a study similar to Aricha et al., [7] in which they found that culture with drug susceptibility testing (DST) had a 100% concordance with LPA while it had only 64.4% concordance

with Xpert MTB/RIF. On analysing the discordant samples between LPA and Xpert MTB/RIF, they found that 91.3% of them matched LPA and culture results while the remaining 8.7% matched Xpert MTB/RIF with culture.[8]

This discordance between two genotypic methods presents a unique diagnostic and therapeutic dilemma. The management of such cases should be determined by clinical judgement. In our patient, LPA was positive on a direct sample and not on a culture sample. The child had grown MRSA on bacterial culture and had responded to antibiotics.

As our patient appeared clinically well, was gaining weight and his surgical site was healing adequately on antibiotic therapy, we considered his LPA as falsely positive due to contamination. Thus, we did not start him on anti-tubercular therapy and instead decided to follow-up monthly.

Compliance with ethical standards

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Conflict of Interest: None

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