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Short Communication

Car windshield fragments as cheap alternative glass beads for homogenization of *Mycobacterium tuberculosis* cultures in a resource-limited setting

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ABSTRACT

Tuberculosis is a global health problem which has been compounded by the emergence and rapid spread of drug resistant strains. Phenotypic drug susceptibility testing of *Mycobacterium tuberculosis* usually requires homogenization of cultures using 3–5 mm glass beads. In resource limited settings, these important material may either not be readily available in the country as in our case requiring that one orders them from abroad or they may be too expensive. In both situations, this would impact on the usually lean budget. In our centre were we recently introduced tuberculosis culture and drug susceptibility testing using the Microscopic Observation Drug Susceptibility (MODS) technique, we successfully used glass fragments from a broken car windshield obtained from a mechanic workshop to homogenize solid cultures to prepare positive controls. All cultures homogenized with these local beads gave consistent MODS results. The challenge of the limited availability of resources for research in resource limited settings can be met by adapting available materials to achieve results.

Introduction

Tuberculosis (TB) is a treatable disease that has resulted in the death of more than 4.6 million people in the last 3 years [1]. The highest burden of disease is borne by developing countries which are also saddled with the challenges of the paucity of resources and relevant infrastructure. Although the World Health Organization (WHO) has observed a gradual decline in the incidence of disease in the last three years, there is an increase in the spread of multi-drug resistant tuberculosis (MDR-TB) [1]. In Nigeria – a high TB burden country with an incidence rate of 204/100,000 population–MDR-TB prevalence gradually rose from 9.7% in 2010 to 13.1% in 2012 [1].

Drug susceptibility testing (DST) of *Mycobacterium tuberculosis* frequently requires manipulation of cultures or specimens with glass beads of 3–5 mm size. In the proportion method of culture and DST, 3 mm glass beads are required to homogenize

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M. tuberculosis cultures to prepare a standard inoculum [2]. Glass beads are also needed for the homogenization of solid culture to prepare inoculum for indirect susceptibility testing in most noncommercial culture and DST methods, such as the Microscopic Observation Drug Susceptibility (MODS) assay [3,4], the Nitrate Reductase Assay (NRA) [5], and Thin Layer Agar (TLA) culture [6]. The mucolytic effect of homogenization of sputum with glass beads in resource-limited settings where N-acetyl-cysteine (NALC) is unavailable has also been reported [7].

Finding this important material required for the work-up of TB is challenging in this environment. The following describes how a substitute was produced using available waste materials.

Materials and methods

Staff visited an automobile mechanic workshop in the environs of the hospital where fragments of glass from broken car windshields were harvested (Fig. 1). Several of the fragments measuring between 3 and 5 mm at the widest diameter were selected (Fig. 2). The glass beads were soaked in 1% sodium hypochlorite (household bleach), washed and then dried in the oven. The beads were sterilized in glass tubes by autoclaving at 121 °C before use (Fig. 3).

The glass beads were used to homogenize clumps of *M. tuberculosis* harvested from Lowenstein–Jensen (LJ) slant cultures to prepare 0.5 McFarland turbidity standard equivalent positive controls which where cultured alongside directly decontaminated sputum as described in the MODS protocol [3]. This was done by harvesting colonies of *M. tuberculosis* from LJ cultures using a sterile loop into a sterile tube containing 100 μL water-tween-80 solution and six sterile glass beads. The tubes were vortexed for 2minutes and allowed to stand for 5 min then vortexed again for 20 s after adding 3 mL of water-tween-80 and allowed to stand for 30 min. The supernatant was transferred to another tube and the turbidity adjusted to 0.5 McFarland turbidity equivalent, which was used for culture. During culture, 5 μL of the 0.5 McFarland equivalent suspensions were added to 5 mL of supplemented Middlebrook 7H9 broth. The preparation was cultured in 24 well tissue culture plates as described for processing positive controls in the MODS protocol [3].

Fig. 1 – Fragmented car windscreen in a mechanic workshop.

Fig. 2 – Separated washed glass beads.

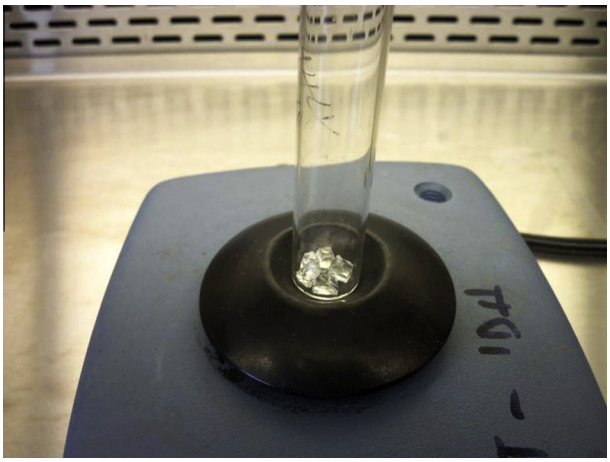

Fig. 3 – Glass beads in a test tube.

Result

All positive control cultures vortexed with the local beads gave consistent MODS microscopy results when compared with the few available imported glass beads at an initial duplicate comparative test run. This was evidenced by a lack of *M. tuberculosis* cords on days 1–4 with strands only seen after growth from day 5 as expected from the protocol (Fig. 4). Thereafter these beads were consistently used to homogenize solid *M. tuberculosis* cultures to prepare positive controls. After use, the glass beads were autoclaved again before discarding in a puncture resistant sharps container.

Discussion

The challenges of carrying out quality research in a resource-limited setting may be daunting. These challenges can

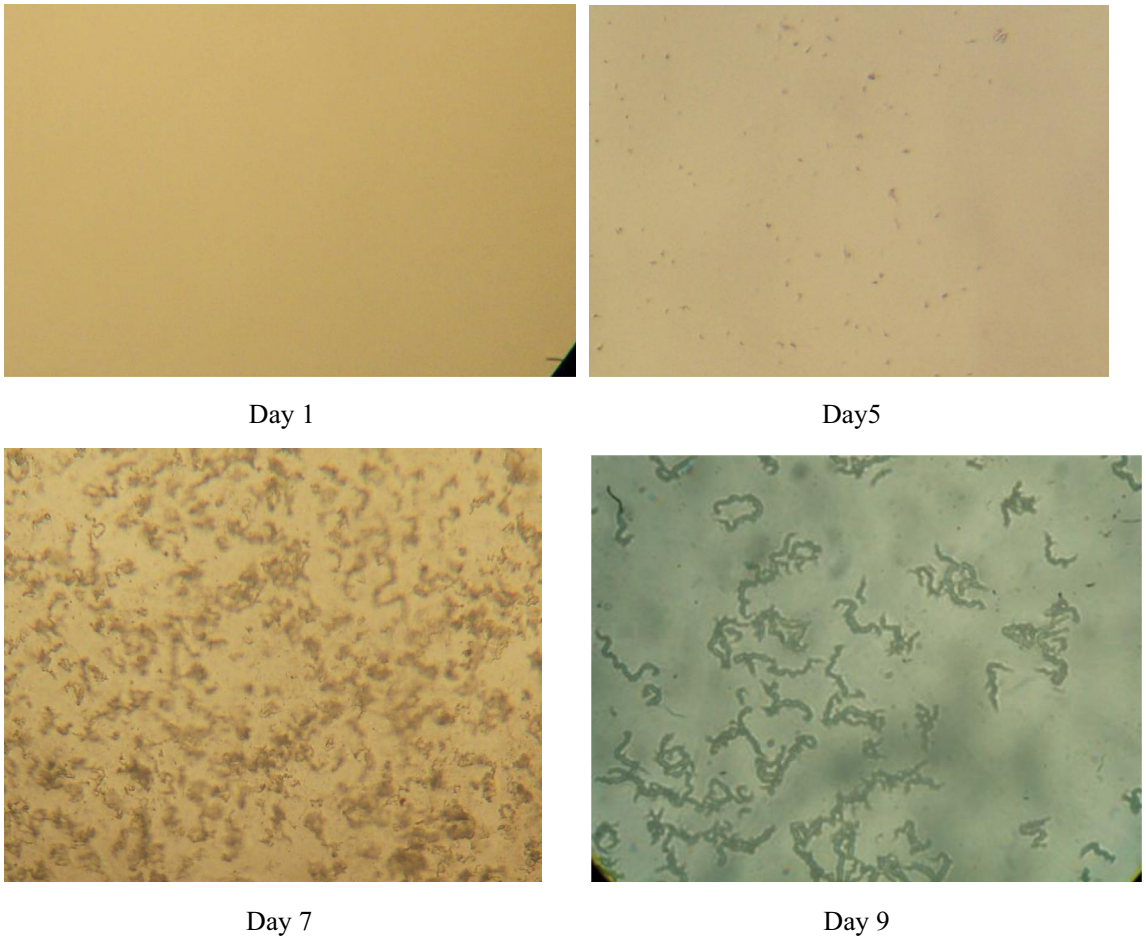

Fig. 4 – Inverted microscopy of MODS cultures of *M. tuberculosis* homogenized with glass beads made from car windshield.

sometimes be surmounted by the ingenuity of scientific researchers as had been demonstrated by a previous study [8]. In most resource-limited settings where TB culture and DST is attempted, the unavailability of glass beads is a limiting factor. Even when available, the cost of purchase may be prohibitive.

However, automobile workshops, referred to as roadside mechanics in this environment, offer an abundance of fragments of car windshields in every nook and cranny of many cities in most developing countries. These glass beads can be collected, washed, disinfected and used as a substitute for imported 3 mm fine glass beads. Where they are larger in size, they could be further fragmented to reduce their sizes. This study found this innovation very useful as it saved both time and money.

Conclusion

This little innovation may also be very useful to TB researchers and laboratory workers in developing economies faced with the same challenges. The reuse of available waste materials will help maximize the funds available for each research budget and allow one to direct these much-needed funds to the purchase of other materials.

Conflict of interest

None.

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