Evaluation of OMNIgene-SPUTUM as a sputum stabilizer for transportation to referral laboratories

Final Project Report

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Background:

Tuberculosis (TB) is one of the top ten leading causes of death worldwide. In Spite of increase in TB detection, still there was a gap of about 2.9 million cases between the notified 7.1 million cases and the estimated 10.0 million (1). Bangladesh is one of the 30 high TB burden countries with an estimated TB incidence of 221 per 100,000 population per year with an estimated 3300 Multi-drug resistant (MDR) TB cases in 2019. The rate of MDR-TB is 0.7% among new cases and 11 % among retreatment cases (1).

Although 292,942 TB cases were detected in Bangladesh in 2019, still we are missing around 68,000 cases as per WHO estimates (2). Lack of a highly sensitive laboratory diagnostic at peripheral health care facilities still remains a challenge which can be an important reason for these missed TB cases (3). Over one thousand microscopy centers are currently functional throughout Bangladesh (4), but, due to low diagnostic yield, TB patients are very often missed to detect by smear microscopy (4). Culture still remains the 'gold standard' for detecting viable *M. tuberculosis* in sputum specimen and also as an indicator of clinical improvement and cure of TB and MDR-TB patients (5). However, it requires advanced laboratory set up along with well-trained technician which is not possible to ensure at all level health facilities in a resource limited country. In Bangladesh, culture is at present available at National TB Reference Laboratories and divisional level specific laboratories (6, 7). For last several years, WHO recommended rapid and highly sensitive real-time PCR (RT-PCR) based technique, Xpert MTB/RIF assay (Xpert) has been widely used to detect *M. tuberculosis* (MTB) within two hours with Rifampicin susceptibility, an important indicator of MDR-TB (8). Although, the National TB Control Program is expanding Xpert facilities over the country, still it is available up to upazilla level health facilities (4). Thus, patients from remote areas are not always getting access to these advanced diagnostics which ultimately hampers timely detection and eventually creates possibility of transmission. An effective sputum transportation mechanism may be a good option to overcome all these challenges.

DNA Genotek Inc., Ottawa, ON, Canada manufactured "OMNIgene-SPUTUM" (OM-S), a transportation reagent, that liquefies and decontaminates sputum and keeps *M. tuberculosis* alive for up to 8 days at as high as 40ºC temperature (9, 10). OM-S treated sputum specimen thus does not require cold chain transportation and is compatible with different conventional and molecular TB diagnostics (11). Studies conducted in different settings (Malawi, Ghana, Uganda) showed comparable Xpert results in OM-S treated and untreated sputum specimens

with standard of care (SOC) transportation (9, 12, 13). OMS is effective at inhibiting the growth of microbial contaminants in sputum and maintain mycobacterial viability even beyond the recommended 8 days although with delayed positivity, showed in previous studies (9, 14). Studies conducted in Nepal evaluated OM-S and showed 9% improved TB detection and 10% less contamination than their existing SOC method but the time to culture positivity was not affected by the processing method (11). Performance investigation from Uganda also showed that OM-S is compatible with microscopy, conventional culture on Lowenstein-Jensen (L-J) and Mycobacterium growth indicator tube (MGIT) (9). From these studies, it is evidenced that OM-S mixed sputum specimen can be used to perform all the currently available TB diagnostics. Improvements in culture positivity and reduced contamination rate in solid culture (L-J) media using OM-S mixed specimen was observed in studies (9, 11). The Technical Expert Group from World Health Organization (WHO) recommended in their report in 2017 to conduct more researches on OM-S to comment on this transport reagent and providing approval (15).

Based on that, we conducted this study to evaluate this reagent in a resource-limited country like Bangladesh, so that it can be used for proper transportation of specimen to the referral laboratories for improved TB detection in this high TB burden country.

Objectives:

Primary Objectives:

1. To evaluate the effect of OM-S as a sputum stabilizer for transportation to referral laboratories
2. To increase the detection of *M. tuberculosis* using OM-S reagent in sputum specimen
3. To compare the contamination rate of OM-S mixed sputum specimen to that of sputum specimen without OM-S
4. To identify the operational challenges using OM-S reagent for sputum transportation

Secondary Objectives:

1. To analyze the direct cost of sputum processing, storage at field site and transportation with and without OM-S reagent in sputum specimen
2. To compare the Xpert results of both centrifuged and non-centrifuged OM-S mixed sputum specimens for a subset of patients

Methodology:

Study design:

This was an exploratory study.

Study sites:

The study was conducted at five health facilities with DOTS centers; four of them situated near to Dhaka city and one facility situated outside Dhaka city.

According to the standard procedure, sputum specimens should be transported to the referral laboratories within 24 hours of collection. Therefore, to evaluate the effect of OM-S reagent on sputum specimens during transportation, we selected four facilities near Dhaka city from where all sputum specimens were transported to icddr,b laboratory within same working day. The field sites near Dhaka city were:

- Keraniganj Upazilla Health Complex
- Narayanganj 300 Bedded Hospital
- Savar Upazilla Health Complex
- Dhamrai Upazilla Health Complex

Besides, to investigate the performance of OM-S for increased detection of MTB in comparison to usual sputum transportation mechanism, we selected one site, Chest Disease Clinic, Chattogram, around 251 sq. km outside Dhaka city from where all sputum specimens were transported to icddr,b laboratory by local courier services. Our laboratory staff member placed at the field site received necessary training on specimen packaging and transportation. A letter of permission was shared with all the field site authorities for conducting the activities at field sites properly.

Staff training

Field (one Field Research Assistant and four Field Assistants) and laboratory staff members were trained properly regarding study activities and their responsibilities. One Field Assistant was placed at each hospital for carrying out field activities and the Field Research Assistant was responsible for supervising these activities.

Photo 1 & 2: Staff training

Enrolment of study participants

The smear-positive PTB patients from the DOTS centers of the selected facilities were enrolled. The Field Assistants collected informed written consent from the adult participants (≥18 years old) and both consent and assent from both guardians and participants who were 15-17 years old. The patients were interviewed about their socio-demographic and clinical status and, their height and weight were measured to calculate body mass index (BMI). The patients were provided with sterile container and they were asked to bring a fresh early morning sputum specimen. Field Assistants then collected sputum specimen from the patients and transported to icddr,b Mycobacteriology laboratory to perform laboratory testing. The Field Research Assistant from the study team visited the field sites frequently to monitor the daily activities of Field Assistants.

Case definition

Smear-positive pulmonary TB patient

The patient diagnosed as PTB patient by smear for AFB microscopy from sputum specimen. Specimen collected at field site and transported to the icddr,b mycobacteriology laboratory.

Participant's criteria:

Inclusion criteria

Smear-positive PTB patients-

- Diagnosed at the DOTS centers in the selected health facilities
- More than 11 years old
- Can expectorate adequate (≥6 mL) sputum specimen

- Consented and assented (where applicable) to be enrolled in this study

Exclusion criteria

Participants, did not match the inclusion criteria, were not enrolled.

Field implementation period:

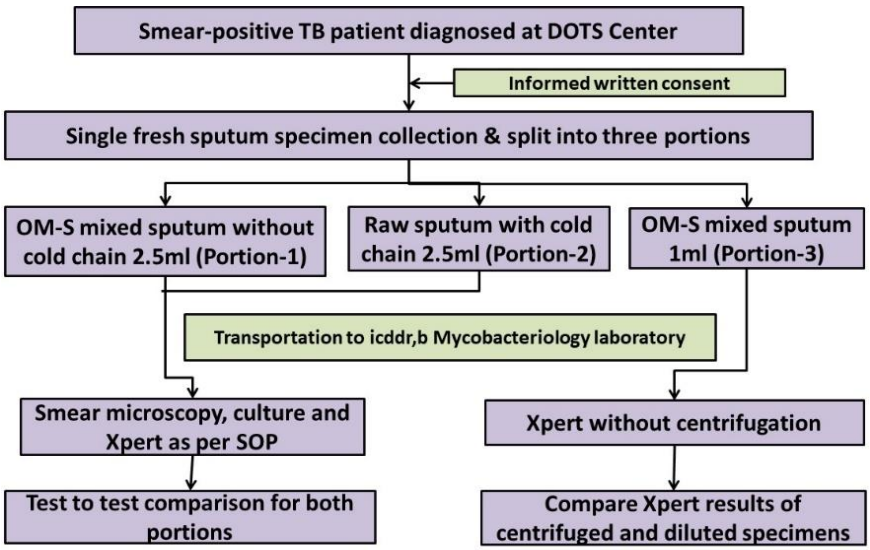
From January 2019 to December 2019.

Sample size:

To evaluate the effectiveness of OM-S as sputum stabilizer; the sample size was not calculated following any formula. We assumed that, we will be able to enroll 15 smear-positive PTB patients matching our study criteria per month on an average from four above mentioned field sites. It was hoped to enroll 200 smear-positive PTB patients from the hospitals near Dhaka city for the first objective.

For second objective, we assumed that we will be able to improve detection of MTB by 10% among the OM-S mixed sputum specimens. With 80% power and 95% confidence interval (CI), sputum specimens from 196 smear-positive TB patients needed to be tested. Considering ~15% drop out or loss of data, the sample size was 225. We have calculated the formula as stated by Tilaki HL (16). Therefore, the total calculated sample size was 425. During the study period, 444 patients were enrolled from the selected hospitals.

Figure 1: Study flowchart



Specimen collection and transportation:

After enrolment, the patients were requested to provide a single sputum specimen in a sterile container provided by the Field Assistant. After collecting the specimen, the Field Assistants split the specimen into two equal and labelled as “portion 1” and “portion 2”. The “portion 1” was mixed with OM-S reagent and the other one “portion 2” was “raw”. The raw sputum specimens (portion 2) was stored in a cool box (with ice-bags) temporarily to maintain the temperature and the OM-S mixed sputum specimens (portion 1) was temporarily preserved in room temperature at field sites. In a subset group, one mL OM-S mixed sputum specimens was separated to another container, labelled as “portion 3” and preserved in same box with “portion 2” in room temperature. Every day the specimens were transported to icddr,b Laboratory for laboratory tests performance.

Photo 3: Taking consent

Photo 4: Data collection

Photo 5: Sample processing

One “temperature monitor” was placed with each sputum transportation box so that the temperature of the specimen while received at the laboratory could be recorded. Upon receiving at the laboratory, the all the specimens were subjected to smear microscopy, culture and Xpert assays. “Portion 3” was tested by Xpert assay only following the same steps mentioned above only “without centrifugation”. The time of specimen collection at field site and their arrival at the laboratory were recorded.

In this study, we also evaluated the operational challenges for using OM-S reagent for instance, difficulty in using OM-S in sputum specimens at field sites, fitness of procurement chain with the shelf life, storage capability in remote field sites etc. A semi-structured questionnaire was used to interview the FAs regarding use and storage of OM-S at field sites. Another questionnaire was used to interview the laboratory staff of icddr.b regarding its usage and procurement issues.

In addition, we have collected data regarding the cost of sputum transportation using OM-S reagent (without cold chain) and that of raw sputum (cold chain transportation) in conventional way from near and distant Dhaka sites.

The following points were considered for cost analysis-

- The cost of the logistics and reagent required for processing and temporary storage of sputum at field site. The items we considered are:
    - ❖ Specimen processing and storage at field site
    - ❖ Ice pack
    - ❖ Freezer maintenance
    - ❖ Cool box (for 6-8 specimens)
    - ❖ Specimen fixing rack (for 8 specimens)
    - ❖ Zip lock bag
    - ❖ Stirrer
    - ❖ Price of OM-S reagent for 1 ml specimen (1:1)
- The cost required for sputum transportation, which was same for transporting both “OM-S mixed” and “Raw” specimens from respective study sites.

Photo 6: Storage of raw sputum

Photo 7: Storage of OM-S mixed sputum

Laboratory procedures:

All sputum specimens (OM-S and raw) from smear positive TB patients were subjected to smear microscopy, Xpert MTB/RIF and microbiological culture tests.

Smear microscopy

After processing of sputum specimens, sediment was used for microscopy. A loop-full of the processed sample was stained by Ziehl-Neelsen (ZN) staining following standard procedure (ref).

Culture and susceptibility testing

Culture and antibiotic susceptibility testing of *M. tuberculosis* strains were performed according to conventional methods (26). The processed sputum specimen was inoculated on 2 Lowenstein Jensen (L-J) slants. The L-J slants were incubated at 37°C for 8 weeks and examined once every week for any growth of visible mycobacterial colony as well as contamination. After getting sufficient culture growth, a standard suspension of *M. tuberculosis* isolates was inoculated onto L-J media containing antimicrobial agents and also onto control L-J media without any antimicrobial agent. Isolates were considered resistant to a particular concentration of drug when 1% or more colonies grow on the drug-containing medium compared to the drug-free medium.

Xpert MTB/RIF Assay

For sputum specimens without OM-S, raw and unprocessed sputum was used for Xpert testing, OM-S mixed specimens were concentrated by centrifugation before Xpert assay. Xpert assay was performed according to the manufacturer's instruction. The test integrates sample processing and PCR in a disposable plastic cartridge containing all reagents required for bacterial lysis, nucleic acid extraction, amplification, and amplicon detection. Sample reagent buffer were added to the specimen in a 2:1 ratio and mixed manually by agitating twice during a 15 minutes of incubation period at room temperature. Then 2 ml of the inactivated sample-buffer mixture was transferred to the Xpert assay cartridge. Cartridges were inserted into the test platform and the automatically generated results were read. Besides, a subset of OM-S mixed sputum specimens was subjected to Xpert assay following the same steps mentioned above only without centrifugation.

Delivery of Laboratory Test Results, TB Diagnosis and Treatment:

Smear positive TB patients diagnosed at the DOTS centres of the selected health facilities were enrolled in this study. Therefore, all the patients received TB treatment at those centres as per the national guideline under the local facility. All the laboratory tests results performed under this study were provided to the local physicians and to the parents/guardians of the patients upon their availability. Rifampicin resistant patients were reported to local physicians and referred to the regional MDR-TB treatment centres (National Institute of Diseases of the Chest and Hospital for Dhaka and, Chest Disease Hospital for Chattogram sites) for treatment according to national guideline.

Statistical analysis:

We analyzed data using data analysis software, Statistical Package for the Social Sciences (SPSS) version 20. We used proportions to summarize the socio-demographic details, and symptom profile. We developed 2X2 tables for each laboratory tests for raw and OM-S mixed sputum specimen. Contamination, that was observed at icddr,b laboratory during specimens' processing for culture for the first time after transportation from field sites, were considered as "primary contamination". Performance of OM-S mixed sputum for each test was compared by concordance and discordance. We compared the difference between two rates by Medcalc online calculator and p value <0.05 at 95% confidence interval was considered as significance. We have also analyzed the sputum transportation cost from near and distant sites to icddr,b mycobacteriology laboratory with and without OM-S reagent.

Results

Baseline characteristics:

A total of 444 patients were enrolled from the respective field sites, of which n=198 from near to Dhaka sites and n=246 from outside Dhaka. Mean age of the patients was 38. 7±16.0 years and the ratio of male was twice than that of female (Table 1). During enrolment, 443 (99.8%) had cough for >2 weeks, 435 (98.0%) had fever for >2 weeks, 322 (72.5%) had significant weight loss and 79 (17.8%) had contact with TB patient in family within last one year (Table 1). The demographic and clinical profiles of TB patients have been detailed in Table 1.

Table 1: Demographic and clinical profile of smear positive TB patients enrolled from selected health care facilities in Dhaka, Bangladesh during January-2019 to December-2019, N=444

Characteristics		Number (N=444)	Percentage (%)
Age			
	Mean age (years): 38. 7±16.0		
Gender			
	Male	298	67.1
	Female	146	32.9
Cough			
	Up to 2 weeks	1	0.2
	More than 2 weeks	443	99.8
Fever			
	No fever	1	0.2
	Up to 2 weeks	8	1.8
	More than 2 weeks	435	98.0
Other symptoms			
	Loss of appetite	415	93.5
	Night sweats	426	95.9
	Significant weight loss	322	72.5
	Haemoptysis	31	7.0
	Chest pain	319	71.8
	Shortness of breath	172	38.7
	Previous history of TB	24	5.4
	History of contact of TB in family	79	17.8

Comparison of laboratory tests between OM-S mixed and Raw sputum specimens:

- ***Same day transportation (from near to Dhaka sites)-***

Among 198 patients enrolled from near to Dhaka sites (same day transportation), smear microscopy was positive on 190 (95.6%) “OM-S mixed” sputum specimens and 192 (96.9%)

"raw" specimens (concordance: 97.9%). Culture was positive on 184 (92.3%) "OM-S mixed" and 186 (93.9%) "raw" specimens (concordance: 96.9%) [Table 2]. The median (inter-quartile range/IQR) time to culture positivity for both "OM-S mixed" and "raw" sputum for the sites near to Dhaka was 35 (28, 42) days. The primary contamination rate of culture in "OM-S mixed" sputum was higher (6.1%) than raw sample (5.6%). All "OM-S mixed" sputum (n=198) specimens were positive on Xpert whereas, 197 (99.5%) "raw" sputum showed positive result (concordance 99.5%) [Table 2].

Table 2: Test-to-test comparison between OM-S mixed and raw sputum specimens collected from the smear positive TB patients enrolled from selected health care facilities situated near to Dhaka, Bangladesh during January-2019 to December-2019, N=444

OM-S mixed	**Raw**			**Positivity Rate**		**Concordance**
	Neg n (%)	Pos n (%)	Total n (%)	OM-S n (%)	Raw n (%)	**(%)**
AFB						
Neg	5 (83.3)	3 (1.6)	8 (4.0)	96.0	97.0	
Pos	1 (16.7)	189 (98.4)	190 (96.0)			**97.9**
Total	6 (100.0)	192 (100.0)	198 (100.0)			
Culture						
Neg	7 (77.8)	4 (2.2)	11 (5.6)	OM-S	Raw	
Pos	2 (22.2)	182 (97.8)	184 (94.4)	94.4	95.4	**96.9**
Total	9 (100.0)	186 (100.0)	195* (100.0)			
Xpert						
Neg	0 (0.0)	0 (0.0)	0 (0.0)	OM-S	Raw	
Pos	1 (100.0)	197 (100.0)	198 (100.0)	100	99.5	**99.5**
Total	1 (100.0)	197 (100.0)	198 (100.0)			

*Three sputum in both portions showed contamination repeatedly, therefore, excluded from analysis

Neg-Negative; Pos-Positive; OM-S- OMNIgene.SPUTUM; Column percentage

- ***Distant transportation through local courier service (from Chattogram site)-***

A total of 246 patients enrolled from CDC, Chattogram. Mean ±SD time spent for transportation from Chattogram to Dhaka was 3.1±1.3 days. Smear microscopy was positive in 239 (97.2%) "raw" and 229 (93.1%) OM-S mixed sputum specimens [Table 3]. Culture showed positive result on 227 (92.3%) "OM-S mixed" and 220 (89.4%) "raw" sputum specimens (increased 3% among OM-S mixed sputum). The median time to culture positivity

for both portions was 35 (28, 42) days. The primary contamination rate of culture in "raw" specimens was significantly higher than "OM-S mixed" specimens (9.8% vs 2.0%; p<0.05). In both "OM-S mixed" and "raw" portions 245 (99.6%) were positive on Xpert and one showed negative (concordance: 100%) [Table 3].

Table 3: Test-to-test comparison between OM-S mixed and raw sputum specimens collected from the smear positive TB patients enrolled from CDC, Chattogram, Bangladesh during January-2019 to December-2019, N=246

OM-S mixed	**Raw**			**Positivity Rate**		**Concordance**
AFB	Neg	Pos	Total	OM-S	Raw	
	n (%)	n (%)	n (%)	n (%)	n (%)	
Neg	3 (42.9%)	14 (5.9%)	17 (6.9%)	93.1%	97.2%	
Pos	4 (57.1%)	225 (94.1%)	229 (93.1)			**92.7%**
Total	7 (100.0%)	239 (100.0%)	246 (100.0%)			
Culture						
Neg	10 (38.5%)	9 (4.1%)	19 (7.7%)	OM-S	Raw	
Pos	16 (61.5%)	211 (95.9%)	227 (92.3%)	92.3%	89.4%	**89.8%**
Total	26 (100.0%)	220 (100.0%)	246 (100.0)			
Xpert						
Neg	1 (100.0%)	0 (0.0%)	1 (0.4%)	OM-S	Raw	
Pos	0 (0.0%)	245 (100.0%)	245 (99.6%)	99.6%	99.6%	**100%**
Total	1 (100.0%)	245 (100.0%)	246 (100.0%)			

Neg-Negative; Pos-Positive; OM-S- OMNIgene.SPUTUM; Column percentage

Operational Challenges Evaluation:

- ***Remarks from field staff members:***

Four field staff was interviewed regarding usage of OM-S at field level. The findings are as follows:

Findings:

- All the respondents mentioned that they did not face any challenges while mixing the OM-S with the sputum specimen.

  "It is very easy to use OM-S and we can take it out easily from the bottle using a dropper. Nothing else is required for processing of specimen with OM-S".

- They mentioned that, sputum mixed with OM-S does not require ice pack for preservation and transportation. Therefore having a fridge at field site is not mandatory.

- In case of raw sample, ice pack is required for storage and transportation of specimens as mentioned by the respondents. There must be supply of electricity and facility of a fridge at field site as the ice pack needs to be stored in a fridge.

  "I have faced difficulties to keep ice pack in the refrigerator of the hospital. As they used to switch the refrigerator off while the office time ends. So my ice packs did not set well in most of the days".

- Temperature of the raw samples needs to be maintained properly said the respondents, otherwise it might hamper the quality of samples which might lead to inaccurate laboratory results.

  "When transporting raw sample, there is always a tension about the ice pack and maintenance of temperature. The ice might melt if it is too hot and if the ice melts, the quality of the sample will be compromised".

- Two of the respondents mentioned that if the sputum is thick, it is difficult to mix the sputum with the reagent and it sometimes consumes more time than usual to be mixed properly with the reagent.

- ***Interpretation:***

- OM-S is user-friendly.
- OM-S mixed sputum can easily be preserved at room temperature.
- Does not require ice pack for preservation and transportation.
- Reagent mixed sputum can be transported from any remote places.
- If sputum is thick, it does not mix well with the reagent and it needs to be shaken more than 20 times for proper mixing.

- ***Remarks from lab personnel:***

Five lab personnel were interviewed to know about any difficulties they faced while processing and performing laboratory tests using OM-S mixed specimens.

Findings:

- All the respondents mentioned that using OM-S mixed sputum specimen is easier to process than raw specimen.

"Conventional sample processing requires more man power, and it requires some extra reagents for decontamination process. But these are not required if OMNIgene is used".

- If the sputum is thick, it does not mix well with the reagent. In that case the sputum and reagent needs to be vortexed more.

Interpretation:

- For OM-S mixed sputum, NALC decontamination steps can be excluded during sample processing- this is time saving and reduces laboratory workload
- For thicker sputum, more vortexing is required for mixing of OM-S perfectly

Cost Analysis of transporting sputum with OM-S and without OM-S:

Considering the cost, we spent for procuring each 250 mL bottle OM-S reagent, required for 250 mL sputum specimen processing in 1:1 proportion, we found that BDT 119.7 is required each one mL sputum specimen processing. We also included the cost of logistics we required for mixing OM-S reagent like stirrer (minimal cost). Based on this, the cost we calculated has been given below:

- ***For same day transportation (near to Dhaka site):***

We required a total of BDT 154.9 for 1 mL OM-S mixed and BDT 37.2 for 1 mL Raw sputum specimen for processing and temporary storage at field site. The transportation cost was similar as they were transported together at same time [Table 4].

- ***For distant transportation (from Chattogram site):***

A total of BDT 155.1 was required for 1 mL OM-S mixed and BDT 37.4 for 1 mL Raw sputum specimen for processing and temporary storage at field site. The transportation cost was similar as they were transported together at same time [Table 4]. Transportation cost has been detailed in Annex.1.

Table 4: Cost of transporting 1 mL sputum with and without OM-S from near and distant Dhaka sites collected from the smear positive TB patients enrolled during January-2019 to December-2019

	Same day transportation from near to Dhaka	**Distant transportation from Chattogram**

Cost category				
	OM-S mixed sputum (BDT)	**Raw sputum (BDT)**	**OM-S mixed sputum (BDT)**	**Raw sputum (BDT)**
Sputum processing and storage at field site (1 mL)	154.9	37.2	155.1	37.4
Sputum transportation (per day per specimen, shared cost	523.6	523.6	523.2	523.2

- *BDT-Bangladeshi Taka*
- *Transportation cost was same for each respective site*
- *For near to Dhaka sites, both ways transportation cost was calculated; for Chattogram, we calculated only courier cost*
- *Since we transported both the cool boxes (one cool box with OM-S mixed specimen without ice pack and other with raw specimen with ice pack) together, so this was a shared cost. Thus the cost was divided by 2 for this study*

Comparison of Xpert MTB/RIF results between diluted and centrifuged OM-S mixes sputum specimens

Among the subset sputum specimens collected for Xpert testing without centrifugation (n=100), 99 (99%) sputum were positive on Xpert with or without centrifugation (concordance 99%). However, one sputum was positive on Xpert with centrifugation and negative while diluted [Table 5].

Table 5: Comparison of Xpert MTB/RIF assay between centrifuged and diluted OM-S mixed sputum sputum specimens collected from the smear positive TB patients enrolled from selected health care facilities situated near to Dhaka, Bangladesh during January-2019 to December-2019, N=100

Diluted	**Centrifuged**			**Positivity Rate**		**Concordance**
Xpert	Neg n (%)	Pos n (%)	Total n (%)	Diluted n (%)	Centrifuged n (%)	n (%)
Neg	0 (0.0)	1 (1.0)	1 (1.0)	99.0	100	
Pos	0 (0.0)	99 (99.0)	99.0 (99.0)			**99.0**
Total	0 (0.0)	100 (100.0)	100 (100.0)			

Neg-Negative; Pos-Positive; Column percentage

Among the subset sputum specimens collected for Xpert testing without centrifugation (n=106), only one sputum was positive on Xpert while centrifuged but error while diluted resulting in an error rate of 0.9%. Of the rest 105 sputum, 104 (98%) sputum were positive on Xpert with or without centrifugation and one sputum was negative in both centrifuged and diluted portions (concordance 99%) [Table 6].

Table 6. Comparison of Xpert MTB/RIF assay between centrifuged and diluted OM-S mixed sputum sputum specimens collected from the smear positive TB patients enrolled from CDC, Chattogram, Bangladesh during January-2019 to December-2019, N=106

Diluted	Centrifuged			Concordance
Xpert	Neg	Pos	Total	
	n (%)	n (%)	n (%)	
Neg	1 (100.0)	0 (0.0)	1 (0.9)	
Pos	0 (0.0)	104 (99.0)	104 (98.1)	**99.1%**
Total	1 (100.0)	105* (100.0)	106 (100.0)	

**One sputum was positive on Xpert while centrifuged but error while diluted*

Neg-Negative; Pos-Positive; Column percentage

Discussions

This is the first study conducted in Bangladesh context where we assessed the effect of OM-S reagent itself on sputum specimen. We also evaluated the effectiveness of OM-S for sputum transportation from distant sites in ambient temperature to inhibit microbial contaminant and recovery of viable TB bacilli. Our major findings revealed that i) OM-S does not have any effect while mixing with sputum specimen; ii) Primary contamination rate of culture was significantly lower in OM-S mixed specimen for distant transportation although positivity rate was not significantly increased; iii) Time to culture positivity was similar in both portions; iv) Xpert MTB/RIF assay results were comparable in OM-S mixed sputum specimen irrespective of centrifugation; and v) OM-S is user-friendly.

In same day transportation arm, we aimed to see whether the tests results showed any discrepancy between "OM-S mixed" and "raw" portions which would indicate the effect of OM-S itself on sputum specimen, being a biochemical reagent. In this arm, we found all the test results to be comparable in both portions, however, minimal reduction of culture positivity (only 1%) in "OM-S mixed" sputum. These results indicate that, OM-S is safe on sputum while mixed with it. We did not find any study specifically commented on its safety on sputum, however, studies conducted on OM-S usage for distant transportation have not reported any side effect on sputum (10, 17).

Our study revealed that the primary contamination rate of culture was significantly lower in OM-S mixed specimen for distant transportation. Similar outcomes were also observed in previous studies where OM-S reduced the rate of contamination on solid culture (18). However,

the culture positivity rate was only increased by only 3% in “OM-S mixed” sputum, which was not significantly increased. This finding was dissimilar to previously conducted studies, where culture was significantly increased in OM-S mixed sputum. The reason of dissimilarity may be due to the mean time to transportation from distant Dhaka which was around 3 days. If we would have selected more distant or remote sites, the positivity rate could have been more in OM-S mixed portion.

Another important observation was, we did not find any delay for culture positivity in “OM-S mixed” portion. The median time to culture positivity was 35 days for both “OM-S mixed” and “raw” sputum and the time was similar for both same day transportation and distant transportation arms. Study conducted in Maputo also didn’t found any significant difference in time to culture positivity for OM-S mixed and raw sputum for same day arm, however, five day arm OM-S treated sputum consumed one week longer time to positivity than raw sputum (11, 19).

Regarding Xpert MTB/RIF assay, we found comparable results in “OM-S mixed” specimen irrespective of centrifugation in both arms. Only one sputum showed error result when ‘diluted’ but was positive when it was “centrifuged”. Although previous study recommended centrifugation of OM-S mixed sputum prior Xpert testing to avoid error results, our study finding suggested that “OM-S mixed” sputum can directly be subjected to Xpert testing which indicates, centrifuge machine is not required at Xpert sites to process OM-S mixed sputum which would be expensive. We also found similar Xpert results in “OM-S mixed” and “raw” portions for both arms (100% positivity each for same day transportation and long period transportation), which showed concordance with previous study as well (9, 12, 13, 20).

We also found lower positivity rate of smear microscopy in “OM-S mixed” sputum in distant transportation arm (decreased by 4.1%). Similar results were also found in studies conducted in Ghana and elsewhere (21, 22).

In Addition, we performed a feasibility analysis on using OM-S at field sites. It revealed, OM-S was user friendly and convenient to use at field site as well as in laboratories. This saved time and reduced laboratory workload as NALC decontamination steps was excluded during sample processing. This was already agreed by the researchers in their studies (22, 23). As feasibility study is not available using this reagent so far, we could not compare the other findings of this current study.

Regarding cost analysis, we have found that, we will need around BDT 155 for processing one mL OM-S mixed sputum which is around four times higher than that of "Raw" one. As each mL OM-S cost BDT 119.7, the cost will also increase with increased quantity of sputum. This has also been mentioned in WHO expert Committee Report (22). However, in case of necessary sputum transportation from remote places to referral laboratories to perform culture and DST, especially to follow up the MDR-TB patients, we can consider using this reagent.

Conclusions

OM-S is an effective reagent for storage and transportation of sputum specimens.. It is user friendly and reduces laboratory work-flow. It can be useful to transport sputum from point-of-care to reference laboratories to perform Xpert and culture with lower contamination rate.

Limitations

- We did not include remote areas in this study
- We did not compare OM-S with any other transportation reagent available like CPC
- Rapid culture MGIT was not performed
- Effectiveness of OM-S in paucibacillary specimens (smear negative pulmonary TB, extra-pulmonary TB etc.) could not be evaluated

Recommendations

. From the current study findings, we can recommend the national program to consider adopting this in their guidance. However, we would suggest future large-scale study in remote areas among the paucibacillary specimens to compare the OM-S to other specimen transportation reagents by using rapid culture, MGIT.

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Annex 1:

Table: Specimen Transportation Cost

Field activity (in months)	11	
Working days per month	26	
Total working days during study period	286	
Category	Calculated cost	Description
Per day number of specimen near Dhaka sites	0.7	Total number of specimen near Dhaka/Total number of working days during study period
Transport cost per day near Dhaka sites for raw and OM-S portions (shared cost)#	362.5	Transport cost from Keraniganj: Taka 800 (both ways by CNG) Transport cost from Narayanganj: Taka 800 (both ways by CNG) Transport cost from Savar: Taka 650 (both ways by CNG) Transport cost from Dhamrai: Taka 650 (both ways by CNG) We calculated an average of the transport cost from all the sites by CNG = (800+800+650+650)/4 =2900/4 = 725 # Since we transported both the cool box (one cool box with OMS mixed specimen and other with raw specimen) together, so this was a shared cost. Thus the cost was divided by 2.
Total transport cost per day per specimen (near)	**523.6**	**Transport cost per day/number of specimen per day**
Per day number of specimen outside Dhaka sites	0.9	Total number of specimen distant to Dhaka/Total number of working days during study period

Transport cost per day distant to Dhaka site for raw and OM-S portions (shared cost)	450.0	Courier cost of 2 cool boxes (one cool box with OMS mixed specimen and other with raw specimen) from CTG to Dhaka: = 250+250 = 500 Courier cost of 2 cool boxes back from Dhaka to CTG: = 200+200 = 400 Total transport cost both ways= 500+400 = 900 Since we transported both the cool box (one cool box with OMS mixed specimen and other with raw specimen) together, so this was a shared cost. Thus the cost was divided by 2.
Total transport cost per day per specimen (distant)	**523.2**	**Transport cost per day/number of specimen per day**