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REVIEW ARTICLE

Should the 'bleach microscopy method' be recommended for improved case detection of tuberculosis? Literature review and key person analysis

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SUMMARY

SETTING: It has been proposed that the sensitivity of direct sputum smear microscopy can be improved if sputum is liquefied with sodium hypochlorite (NaOCl or household bleach), and concentrated by centrifugation before acid-fast staining.

OBJECTIVE: To summarise the results of the studies of the bleach method for improved sensitivity of sputum microscopy and to describe the opinions and knowledge of key persons in National Tuberculosis Control Programmes (NTPs) about this method.

DESIGN: We searched Medline, EMBASE and Web of Science for studies comparing the bleach method to direct sputum smear microscopy in low- or middle-income countries. Each study was assessed regarding methodology and field applicability. We also sent out questionnaires concerning the bleach method to key persons in NTPs in 85 countries.

RESULTS: In 15 of the 19 studies identified there was a statistically significant improvement in the proportion of positive tests *or* sensitivity ranging from 7–253%. The majority (73%) of the key persons had heard of the bleach method. Forty-four per cent thought it could improve case detection in their countries, while 49% did not know; 93% of them would promote the bleach method; the most common reasons for doing so would be recommendations from the WHO or the IUATLD, or favourable studies performed in their own country. The bleach method was used routinely in only three countries.

CONCLUSION: There is enough evidence to recommend the evaluation and introduction of the bleach method in most settings where mycobacterial culture is not performed routinely.

KEY WORDS: tuberculosis; diagnosis; sputum; microscopy; sodium hypochlorite

BACTERIOLOGICAL DIAGNOSIS of tuberculosis (TB) worldwide is to a large extent dependent on direct microscopy of sputum smears after Ziehl-Neelsen (ZN) staining. This method is rapid, specific and reasonably easy to perform, but its sensitivity is not optimal when used in control programmes.[1] As it is likely to diagnose the most infectious patients, the World Health Organization (WHO) nevertheless recommends it for screening patients with cough lasting for more than 2 weeks for tuberculosis infection.[2] Nevertheless, only 42% of 3.8 million notified cases were detected by microscopy in 2001.[3] Mycobacterial culture on solid media is sensitive and specific, but it takes weeks to perform and requires biosafety level III laboratories. It is therefore usually only performed on selected cases (if at all) in countries with limited resources. Newer molecular techniques, such as polymerase chain reaction (PCR), although rapid, are also too costly to be used routinely in those settings where most TB cases occur.

Microscopy clearly has many advantages when it comes to speed and feasibility, and if sensitivity could be improved it has the potential to become an even more valuable tool for National Tuberculosis Control Programmes (NTPs) around the world. In the last decade many researchers have suggested that the performance of sputum smear microscopy can be significantly improved if sputum is liquefied with one or other chemical reagent and then concentrated by centrifugation or sedimentation prior to acid-fast staining. This is well known, and is described in many major textbooks and laboratory manuals.[4,5] The most widely studied procedure is liquefaction of sputum with sodium hypochlorite (NaOCl), usually known as household bleach. Briefly, sputum is mixed with an equal amount of ≈5% NaOCl, and the mixture is incubated at room temperature for 10–15 min. Distilled

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water is added and the sample is concentrated, either by centrifugation for 15 min or by sedimentation overnight. The NaOCl reagent has the advantage of being available almost everywhere. Moreover it is an effective disinfectant, which kills off *Mycobacterium tuberculosis*,[6] and thus probably improves safety in laboratories that lack adequate biosafety facilities. NaOCl is also effective against the human immunodeficiency virus (HIV),[7] and although most HIV particles in sputum are non-infectious it might be of at least psychological importance to laboratory staff, especially if the sputum is haemoptoic.

In spite of the potential benefits, concentration techniques have—somewhat to our surprise—not made it into routine practice in resource-poor settings. It is unclear whether this is because the published studies are not convincing enough, their design is not relevant for the setting where the technique is supposed to be used or the results of the studies are not well known among people working in NTPs.

To address these issues we performed a study with a two-fold aim: 1) to summarise the results of studies of the sodium hypochlorite concentration method (hereafter called the bleach method), with special interest in i) comparisons with culture and direct smear microscopy, and ii) the method's applicability in routine conditions; and 2) to ascertain what key persons working in the NTPs know and think about the bleach method.

To achieve these aims, we reviewed the scientific literature on the topic and we sent out questionnaires to key persons in NTPs worldwide.

MATERIALS AND METHODS

Literature review

We searched Medline, EMBASE and Web of Science for relevant studies (in all languages) using the search terms tuberculosis combined with NaOCl, hypochlorite or bleach. All studies in all languages where the bleach method was compared to direct smear and/or culture in a low- or middle-income country (country classifications according to the World Bank in 2001*) were included. We then checked relevant references, and thereafter those references cited that were relevant to our study. Each study was assessed regarding methodology and relevance for field applicability. If necessary, we tried to contact the authors for clarifications. The results were scrutinised, and as in a few studies inappropriate statistical methods had been used, we recalculated the *P* values using McNemar's test for matched data and Fisher's test for comparing groups. We also calculated 95% confidence intervals for differences in sensitivity or ratio of positive tests with the bleach method compared to the direct method.[8]

Key person analysis

We included countries with a population of more than 1 000 000 (according to 1999 UN estimates†), an estimated TB incidence of more than 10/100 000 population and at least partly applied DOTS at the time of initiating this study.[9] The contact details of the NTP managers and National Reference Laboratory (NRL) managers were requested from the persons in charge of tuberculosis at the six regional WHO offices. A questionnaire was sent to all potential participants by mail, fax and/or e-mail, together with an introductory letter explaining that participation was voluntary and anonymous, and an information sheet presenting the bleach method with references to selected studies. The questionnaire was translated into Russian and Spanish. If no response was received, a reminder was sent out, and if there was still no response, we tried to reach the person in question by telephone. At the time of the reminder, a translation into French was used in the relevant countries. The completed questionnaires were entered and analysed using Epi Info 2002 (CDC, Atlanta, Georgia, USA). If two questionnaires were received from one country (i.e., from both the NTP and NRL managers), answers dealing with specific conditions for that country (e.g., in which settings smear microscopy is performed) were combined. Answers specific to the individual, such as opinions about the bleach method, were treated individually. Information regarding World Bank economic classification, TB incidence and DOTS coverage were also entered and analysed per country.

RESULTS

Literature review

Briefly, 19 distinct studies were found in 13 papers.[10–22] For the purpose of optimal clarity, we chose to treat the results as two distinct studies if the direct method was compared to the bleach method using concentration by centrifugation *and* sedimentation on the same samples.

The methodology of the studies is presented in Table 1. In six studies,[11,13–15,17,21] the bleach method was compared to the direct method *and* to a reference method (gold standard). In these studies it was thus possible to calculate sensitivity and specificity. In 13 studies,[10–12,15,16,18–20,22] the bleach method was compared to the direct method without using any reference method. Consequently, sensitivity and specificity cannot be calculated for these studies; it is only possible to compare the proportion of positive tests.

Nine studies[10,12,13,16,17,19,21,22] compared the number of patients diagnosed with each method (166–948 patients), while the performance of each method was compared on individual samples in 10 studies (100–

* www.worldbank.org

† www.who.int

Table 1 Methodology of 19 studies in which the bleach method was compared to the direct method

Reference	Setting and methodology	*n*	Definition of positive	Blinded	Gold standard
Allwood et al.[10]	324 adult PTB suspects; 1 bleach smear compared to 3 direct smears; consecutive out-patient TB suspects (⩾15 years of age) in hospitals; Malawi and Malaysia.	324 patients	IUATLD	Yes	None
Angeby et al. (I)[11]	265 in- or out-patients with suspected or known PTB at a pulmonary reference hospital, Honduras.	303 samples	IUATLD	Yes	Culture and clinic
Angeby et al. (II)[11]	An unknown number of in- or out-patients with suspected or known PTB at a pulmonary reference hospital, Honduras.	971 samples	IUATLD	Yes	None
Angeby et al. (III)[11]	An unknown number of patients with suspected or known PTB attending urban local health centres, Honduras.	1422 samples	IUATLD	No	None
Aung et al.[12]	New and retreatment cases from a TB institute in Myanmar; laboratory procedures performed at a department of medical research.	948 patients	WHO	Not stated	None
Bruchfeld et al.[13]	Consecutive adult out-patient PTB suspects at a teaching hospital, Ethiopia; 3 direct smears done at the hospital lab; pooled sputum from these 3 samples examined by bleach method at a research institute.	509 patients	WHO	Yes	Culture
Farnia et al.[14]	Sputums from referred or hospitalised patients at a national tuberculosis research institute in Iran. Bleach concentration by sedimentation overnight.	430 samples	IUATLD	Yes	Culture
Gebre et al. (I)[15]	PTB suspects from a TB out-patient clinic, Ethiopia.	100 samples	Not stated	Yes	Culture
Gebre et al. (II)[15]	PTB suspects from a college of medical sciences, Ethiopia.	500 samples	Not stated	Yes	None
Gebre et al. (III)[15]	PTB suspects from a medical college, India.	103 samples	Not stated	Yes	None
Gebre-Selassie (cen)[16]	1 sputum from each adult out-patient PTB suspect at a university hospital in Ethiopia. Bleach concentration performed by centrifugation.	200 patients	Not stated	Not stated	None
Gebre-Selassie (sed)[16]	1 sputum sample from each adult out-patient PTB suspect at a university hospital in Ethiopia. Bleach concentration performed by sedimentation overnight.	200 patients	Not stated	Not stated	None
Habeenzu et al.[17]	2 sputum samples from each of 488 patients attending a university hospital, Zambia. Bleach and direct method performed on each sputum.	488 patients	Not stated	No	Fluorescent microscopy
Miorner et al. (cen)[18]	PTB suspects from a TB out-patient clinic, Ethiopia. Bleach concentration performed by centrifugation.	545 samples	Not stated	Yes	None
Miorner et al. (sed)[18]	PTB suspects from a TB out-patient clinic, Ethiopia. Bleach concentration performed by sedimentation overnight.	545 samples	Not stated	Yes	None
Saxena et al.[19]	PTB suspects from a hospital, India.	304 patients	Not stated	Not stated	None
Van Deun et al.[20]	Consecutive sputum samples from rural TB centres, Bangladesh. Direct smears read there. Bleach smears read at the central laboratory. Concentration performed by sedimentation.	3287 samples	ATS	Yes	None
Wilkinson and Sturm[21]	Consecutive PTB suspects at a district hospital, South Africa. One sample was used for direct microscopy and another for bleach preparation.	166 patients	Not stated	Not stated	Culture
Yassin et al.[22]	Consecutive PTB suspects at a missionary health centre in Ethiopia. 1 standard smear vs. 1 bleach smear concentrated by sedimentation for 30–45 min.	200 patients	IUATLD	Yes	None

PTB = pulmonary tuberculosis; IUATLD = International Union Against Tuberculosis and Lung Disease; WHO = World Health Organization; cen = centrifugation; sed = sedimentation method; TB = tuberculosis; ATS = American Thoracic Society.

3287 samples).[11,14,15,18,20] All studies[10–22] compared both microscopy methods (and a gold standard method, if applicable) on each sample or patient; i.e., no study compared one method on one group of samples or patients with another method applied on a different group of sample or patients.

In 13 studies, the samples or patients were recruited from hospitals, medical colleges or TB in-

stitutes,[10–17,19,21] in two studies from local health centres,[11,20] in two other studies from a TB out-patient clinic,[15,18] and in one study patients were recruited from a missionary health centre.[22] One study compared the diagnostic performance of the bleach method to the standard three-smear strategy.[10] Another study compared the direct three-smear strategy to the bleach method on the same three samples pooled.[13] In two studies the bleach smears were concentrated by sedimentation (45 min and overnight, respectively),[20,22] while in two papers, concentration by sedimentation overnight was compared to centrifugation and to direct smears.[16,18] In nine studies, the definition of what is considered as positive was stated.[10–14,20,22] In 13 studies, the readings were blinded, i.e., it was stated in the paper that the reader(s) were not aware of the results of one method when reading slides performed with the other.[10,11,13–15,18,20,22]

The results of the studies are presented in the Figure and Table 2. Enough data were presented for 15 studies to be able to analyse matched samples or patients with McNemar's test,[10–16,20–22] whereas for four studies we had to perform the calculations using Fisher's test as if we were comparing two distinct groups.[17–19]

In the six studies where a gold standard was used,[11,13–15,17,21] there was a statistically significant ($P < 0.05$) improvement in sensitivity with the bleach method as compared to the direct method in five, ranging from 16–125%,[11,13–15,17] while one study did not show any improvement.[21] Specificity ranged from 96 to 100% with the bleach method and from 95 to 100% with the direct method.

In 13 studies where no gold standard method was used,[10–12,15,16,18–20,22] there was a significant increase ($P < 0.05$) in the proportion of positive tests with the bleach method as compared to the direct method in 10, ranging from 7 to 253%,[10–12,15,16,19,20,22] while the proportion of positive tests did not differ significantly in three studies.[11,18]

In the two papers where concentration by sedimentation was compared to centrifugation there was a greater although not statistically significant increase in the proportion of positive tests using centrifugation.[16,18]

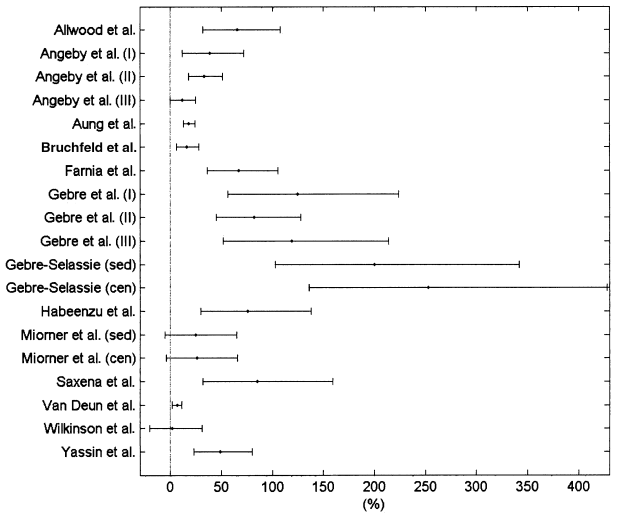

Figure 95% confidence intervals for the relative improvement in percentage of sensitivity or ratio of positive tests with the bleach method compared to the direct method. Above 0% favours the bleach method. sed = sedimentation method; cen = centrifugation.

Key person analysis

A total of 94 countries were eligible according to the inclusion criteria. Contact details were not received for 6/6 countries in the Western Pacific Region (WPRO), 2/27 countries in the African Region (AFRO) and 1/20 countries in the American Region (AMRO). Thus, questionnaires were sent to NTP managers and NRL managers in a total of 85/94 eligible countries. In most cases, the contact details of the NRL manager were not available and the NRL questionnaire was sent via the NTP manager. Answers were received from 84 key persons in a total of 69/85 countries (81%). There was no significant difference regarding response rate or type of responding key person (NTP or NRL manager) between the different WHO regions.

Country-based analysis showed that 80% of the responding countries had an estimated TB incidence of more than 50/100 000/year, 52% more than 100/100 000/year and 22% more than 300/100 000/year. Forty-five per cent of the responding countries were classified as low-income countries and 52% were classified as middle-income countries. Not surprisingly, there was a significant association ($P < 0.0001$) between low economic classification and high TB incidence. Sixty-eight per cent of the countries had a treatment success rate of more than 70%, but only 30% of the countries had a DOTS detection rate (DDR) of more than 50%. Smear microscopy was widely performed in all kinds of settings, while mycobacterial culture was not performed in 6/69 countries (9%), and if performed, it was mostly at a national or regional level or at university hospitals.

The bleach method was used to different extents for routine diagnosis in three of the 69 countries (4%). In one country in the African Region it was used at national, regional and district level, in another country in the European Region it was used at national and regional level and in yet another in the American Region the method was only used in laboratories dealing with sputum from HIV/AIDS (acquired immunedeficiency syndrome) patients in one big city. In two of the countries the key person thought that the method had contributed to an increase in case detection, while in one country the key person did not answer this question.

About three quarters of the 84 responding key persons had heard about the bleach method previously,

Table 2 Results of 19 studies in which the bleach method was compared to the direct method

Reference	*n*	Bleach/direct *n*						Ratio of positives	% Sensitivity	% Specificity	Bleach vs. direct % increase	Statistical method	*P*
		Total positive	Total negative	True positive	True negative	False positive	False negative						
Allwood et al.[10]	324 patients	48/29	276/295	—	—	—	—	15/9	—	—	66	McNemar	<0.0001
Angeby et al. (I)[11]	303 samples	—	—	37/27	241/242	2/1	23/33	—	62/45	99/100	37	McNemar	0.0034
Angeby et al. (II)[11]	971 samples	100/75	871/896	—	—	—	—	10/8	—	—	33	McNemar	<0.0001
Angeby et al. (III)[11]	1422 samples	37/33	1385/1389	—	—	—	—	2.6/2.3	—	—	12	McNemar	0.125
Aung et al.[12]	948 patients	293/248	655/700	—	—	—	—	31/26	—	—	18	McNemar	<0.0001
Bruchfeld et al.[13]	509 patients	—	—	106/91	328/331	14/11	62/77	—	63/54	96/97	16	McNemar	0.0015
Farnia et al.[14]	430 samples	—	—	60/36	355/353	3/5	12/36	—	83/50	99/99	67	McNemar	<0.0001
Gebre et al. (I)[15]	100 samples	—	—	36/16	48/48	0/0	16/36	—	70/31	100/100	125	McNemar	<0.0001
Gebre et al. (II)[15]	500 samples	71/39	429/461	—	—	—	—	14/8	—	—	83	McNemar	<0.0001
Gebre et al. (III)[15]	103 samples	35/16	68/87	—	—	—	—	34/16	—	—	119	McNemar	<0.0001
Gebre-Selassie (sed)[16]	200 patients	51/17	149/183	—	—	—	—	26/8	—	—	200	McNemar	<0.0001
Gebre-Selassie (cen)[16]	200 patients	60/17	160/183	—	—	—	—	30/8	—	—	253	McNemar	<0.0001
Habeenzu et al.[17]	488 patients	—	—	116/66	336/336	0/0	36/86	—	76/43	100/100	76	Fisher	<0.0001
Miorner et al. (sed)[18]	545 samples	114/91	431/454	—	—	—	—	21/17	—	—	25	Fisher	0.0753
Miorner et al. (cen)[18]	545 samples	115/91	430/454	—	—	—	—	21/17	—	—	26	Fisher	0.0640
Saxena et al.[19]	304 patients	96/52	208/252	—	—	—	—	32/17	—	—	85	Fisher	<0.0001
Van Deun et al.[20]	3287 samples	544/510	2743/2777	—	—	—	—	17/16	—	—	7	McNemar	0.0028
Wilkinson and Sturm[21]	166 patients	—	—	39/38	75/73	2/4	50/51	—	44/43	97/95	3	McNemar	1.0000
Yassin et al.[22]	200 patients	52/35	148/165	—	—	—	—	26/18	—	—	49	McNemar	0.0001

sed = sedimentation method; cen = centrifugation.

Table 3 Key persons' answers to the questions

Question	Answer, *n* (%)
Had you heard of the bleach method before receiving the information from us? (*n* = 84)	61 (73) Yes 23 (27) No
If 'yes', specify (*n* = 61)	22 (36) Colleague 29 (48) International scientific journal 4 (7) National scientific journal 17 (28) Conference 16 (26) Other 2 (3) Do not know
Is the bleach method in routine use in your country? (*n* = 84)	3 (4) Yes 81 (96) No
If not in use, could it—in your opinion—improve case detection in your country (*n* = 81)	36 (44) Yes 5 (6) No 40 (49) Do not know
What would make you promote the introduction of the bleach method for routine use in your country? (*n* = 81)	51 (63) WHO recommendations 42 (52) IUATLD recommendations 38 (47) More scientific evidence 50 (62) Studies in my own country 8 (10) Other 3 (4) Would not promote it 3 (4) Do not know
What extra resources would be needed to implement the bleach method in your country? (*n* = 81)	58 (72) Additional training of laboratory staff 50 (62) Additional equipment 47 (58) Additional reagents 6 (7) Other 10 (13) Do not know

WHO = World Health Organization; IUATLD = International Union Against Tuberculosis and Lung Disease.

most of them from an international scientific journal—predominantly the International Journal of Tuberculosis and Lung Disease (IJTLD) (Table 3). Six per cent did not think that the bleach method could improve case detection in their countries, whereas 44% thought it could and 49% did not know. The most important factors that would make the key persons promote the introduction of the bleach technique were WHO and/or International Union Against Tuberculosis and Lung Disease (IUATLD) recommendations, and studies in their own countries. Three responders would not promote it. Most key persons thought they would need additional equipment (mostly centrifuges) and additional reagents as well as additional training of laboratory personnel to be able to implement the new method.

DISCUSSION

The first aim of our study was to summarise the results of studies dealing with the bleach method. We found overwhelming evidence that the bleach method can improve case detection of pulmonary tuberculosis. As some studies were based on the comparison of individual patients and some on individual samples, and as different definitions of positive tests were used, we performed no meta-analysis, but we still feel that the confidence intervals (Figure) speak for themselves. The main questions are: 1) is the improvement good enough to justify the extra cost and labour?, and 2) is the new method applicable under field conditions in settings with limited resources? We feel that the answer to both of these questions is 'yes', but with certain reservations.

This review deals only with the bleach method. Other concentration methods have been studied,[11,14,23–25] but none of them shows better performance and none disinfects the sample. The bleach method is, moreover, the most widely studied method in low- and middle-income countries. For these reasons we chose to limit the review to this technique.

In all of studies identified, the two microscopy methods were evaluated using the same samples. In spite of this, in many studies statistics were calculated using the χ^2 test as if two different groups were compared. Although this method is more conservative we do not think it is fair to the data, as important differences might be lost. We therefore recalculated the statistics where possible, using McNemar's test for matched data.

The ZN technique was used in all studies to stain both direct and bleach-treated samples. However, in the reviewed papers the exact staining method was not described (i.e., hot or cold method, concentration and time of exposure to the reagents, etc.), and instead a reference was given. We therefore cannot know if there is any association between different ZN variants and the extra benefit of bleach.

The bleach method has some built-in disadvantages. First, a bleach-treated sample cannot be used for mycobacterial culture, as the NaOCl kills *M. tuberculosis*. In case mycobacterial culture is asked for, another sample must be requested. On the other hand, this disinfectant potential will increase biosafety in laboratories and may be even more important in settings with a high incidence of HIV, where a high proportion of laboratory staff could be HIV-infected and thus be more susceptible to tuberculosis. It must be recognised, however, that if safety cabinets are not available, the bleach should be mixed with the sample in the container in which it has been deposited—to avoid aerosol formation one should not pour potentially infected sputum into a tube outside a safety cabinet. Although NaOCl is probably one of the most commonly used mycobacterial disinfectants, it has been hard to find exact data on the concentrations and exposure times needed. According to Kent and Kubica, 0.1–0.5% is enough to kill *M. tuberculosis*, but the time needed is not stated.[5] Best et al. showed that a 1% concentration of NaOCl is enough to kill *M. tuberculosis* in sputum in 1 min.[6] The concentration and exposure times in the reviewed papers (i.e., about 2% for about 15 min) should therefore be sufficient to effectively disinfect the specimens.

Second, the bleach method requires more work and more equipment, particularly if concentration is performed by centrifugation. Bleach itself is inexpensive and readily available almost everywhere. The half-life of NaOCl is about 12 months; it is likely to be reduced by 1 month if the bottle is opened and by about 3 months if the ambient temperature is high (around 30°C). These factors must be taken into consideration when planning to use the bleach method in routine. Centrifuges were the equipment most commonly mentioned as lacking by the responding key persons. Studies have shown, however, that enough centrifugal force can be obtained by simple table-top centrifuges that are available in many laboratories for serum and urine analysis.[15] The need for screw-tapped tubes can be bypassed, as the sample is disinfected. Extra work can be reduced if sedimentation is used instead of centrifugation.[16,18,20,22] Yassin et al. could optimise the technique to the extent that they mixed the bleach and the sputum directly in the patients' sputum containers and sedimented it for 45 min simply by inclinating the sputum container by 45°.[22] It should be noted, however, that the use of sedimentation has been less well studied than centrifugation, and that more research might be needed to clarify its role. Also, if one bleach sample can be used instead of three direct smears,[10] or if the three samples are pooled before liquefying with NaOCl,[13] the workload might even be reduced using the bleach method compared to the direct method. Another aspect in favour of the bleach technique is that the lack of debris on the microscopy slide makes reading simpler, and hopefully less time consuming than the 5 min recommended for reading the direct smear.[26] The exact quantity of extra labour and cost will vary from setting to setting, and needs to be estimated locally in further studies.

Third, prolonged exposure to NaOCl (≥60 min) gradually reduces the possibility of detecting acid-fast bacilli.[22] This factor must be acknowledged, especially as in peripheral settings laboratory technicians usually have tasks other than sputum microscopy and it might sometimes be difficult to keep track of time.

Fourth, in all studies but one,[22] distilled water was added after the bleach reaction. It has been suggested that too high a bleach concentration could possibly interfere with ZN staining; however, the exact role of distilled water is not clear. Apart from being an 'extra step,' distilled water may be more difficult to obtain than bleach in many settings. Furthermore, as demonstrated by Yassin et al.,[22] it might not be necessary. It could probably also be replaced by water cleaned by filtration or even ordinary tap water if it is shown not to contain enough mycobacteria to be detected by microscopy. This remains to be studied further.

Finally, the bleach sediment, due to the reduction of debris, can be difficult to see macroscopically on the slide. This might explain Wilkinson and Sturm's results, where many samples that were positive with the direct method were negative with the bleach method.[21] It may be necessary to clearly mark on the slide where the bleach sediment has been put to avoid this problem.

Most studies were performed in hospitals, ranging from district hospitals to national tuberculosis reference hospitals or similar. Only three studies were performed in local health centres. In one study, no significant improvement of case detection was seen,[11] in another, there was a small (7%)—although statistically significant—improvement in case detection,[20] and in yet another, in a missionary health centre, a statistically significant and important improvement of case detection (49%) was seen.[22] Those studies, performed in hospitals, predominantly among out-patients, mainly showed a statistically significant and important improvement in case detection (and sensitivity if applicable) with the bleach method compared to the direct smear technique. The reasons for this difference might be that the quality of the samples is different in patients attending health centres, and/or that a study is more difficult to monitor in peripheral health centres, especially if there are many of them.[11,20] Nonetheless, as the new method seems to be applicable in hospitals, it should be used there, especially because in most settings mycobacterial culture is not performed or is performed only on selected cases. If implementation of the bleach method proves successful in hospitals in a country or region, it might then be reasonable to try to implement it also in peripheral health centres.

The successful implementation of the bleach method should improve case detection and thereby reduce tuberculosis incidence in the long run. Using a mathematical model described by Dye et al., we can see that, assuming 85% cure rate and a 50% detection rate under DOTS (DDR), a 30% increase in DDR would lead to an increased yearly drop in TB incidence rate of less than 9–11%, and a 50% increase in DDR would further increase the drop in the incidence rate to 12%; i.e., an increased drop in the incidence rate of more than 30% per year.[27] Although mathematical models are highly theoretical and it would be speculative to interpret the impact in absolute numbers, it is doubtless that an increase in DDR would be of great importance to a population, and we believe that the implementation of the bleach method could contribute significantly to such an increase. According to the WHO, improvements in case detection should take place only when the cure rate is more than 70%.[2] In our study, however, 68% of the countries fulfilled this condition.

The second aim was to ascertain what people working in the NTPs knew and thought about the bleach method. This is particularly important, as very little is known about how research becomes action and policy.[28] We received answers from 84 key persons, representing 69 (81%) of the countries included.

In many cases with only one respondee, it was understandable from the answer that it had been prepared by both key persons together, or that one of them (usually the NTP manager) had delegated the task to the other.

A clear majority (73%) of the responders had heard of the bleach method previously, mostly from international scientific journals (48%) (predominantly the IJTLD), colleagues (36%) or conferences (28%) (predominantly IUATLD conferences). Despite this knowledge, the method was used routinely in only three countries. Forty-four per cent of the key persons thought the new method could improve case detection in their countries and 49% did not know. Most key persons would promote the introduction of the bleach method if they were recommended to do so by the WHO (63%) or the IUATLD (52%), or if they had performed successful (authors' interpretation) studies in their own countries (62%). Only 4% would not promote it. These figures show that recommendations from relevant international organisations can have a big impact and that there is a considerable eagerness to learn from studies carried out locally. The latter is important, since shift of policy without 'ownership' may cause insufficiency or malfunction in implementation.[29] When asked about what would be needed to implement the new method, additional training of laboratory staff was the most often mentioned extra resource (72%). As the bleach method uses the same staining method (ZN) and the same reading method (ordinary light microscopy), this task seems manageable. The need for additional reagents (bleach), declared by 58% of the responders, also seems to be controllable; virtually all studies found in the review claim that this reagent can be purchased locally almost everywhere, and it is supposedly cheap. More important to take into consideration is the stated need for additional equipment (62%), mostly centrifuges and tubes. Simple table-top centrifuges should be enough to perform the bleach concentration, as there is no need for a high centrifugal force[15] and the sample is disinfected by the bleach. These kinds of centrifuges (and tubes) are available even in many modest laboratories to perform serum and urine analyses. If it is necessary to buy them, the extra cost must be balanced against the potential benefits of introducing the new technique. To our knowledge, no such cost-benefit analysis has been done. However, the cost may be considered a one-time investment. If centrifuges are not available, the use of sedimentation instead may be as satisfactory,[16,18,20,22] although it has been less studied.

Interestingly, the bleach method was not used in routine in any of the countries where the studies had been performed. The Council on Health Research for Development (COHRED) has investigated how research becomes action and policy, and which factors can be changed to improve this process.[28] First, various stakeholders (researchers, research users, policy makers, etc.) should be involved at all the different steps in the course of planning, management and dissemination. Researcher driven research, regardless of how appropriate, well-timed and careful it is, might not lead to action if it is regarded as being imposed upon the research users from outside or even above. Mediators are persons or institutions with the right connections and capabilities who are involved in promoting connections between the research and policy processes, for example national research coordinating bodies or international organisations. According to the COHRED study, mediators play perhaps the most crucial role in the progression from research to action.[28] According to our results, the WHO and the IUATLD, which seem to have a high credibility globally among key persons in NTPs, could play such a role in advocating a new laboratory technique.

CONCLUSION

We conclude that there is enough evidence and enough local concern to promote the introduction of the bleach method as a part of the DOTS strategy in countries where culture is not performed routinely. Relevant organisations such as the WHO and the IUATLD should start to advocate this technology shift. The implementation should involve or be conducted by local health authorities, and should include an assessment of the local laboratory infrastructure. A country-specific evaluation should precede full-scale introduction.

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RÉSUMÉ

CONTEXTE : On a prétendu que la sensibilité de l'examen microscopique direct des frottis d'expectoration peut être améliorée lorsque l'expectoration est liquéfiée par l'hypochlorite de sodium (NaOCl ou eau de Javel) et concentrée par centrifugation avant la coloration pour les bacilles acido-résistants.

OBJECTIF : Résumer les résultats de la méthode au blanc de lessive pour améliorer la sensibilité de l'examen microscopique de l'expectoration et décrire l'opinion et les connaissances de personnes-clé dans les programmes nationaux de lutte antituberculeuse (PNT) au sujet de cette méthode.

SCHÉMA : Nous avons recherché, dans Medline, EMBASE et Web of Sciences, les études comparant la méthode au blanc de lessive et l'examen microscopique direct des frottis d'expectoration dans les pays à revenus faibles ou moyens. Chaque étude a été évaluée en ce qui concerne sa méthodologie et son applicabilité sur le terrain. Nous avons ensuite envoyé des questionnaires concernant la méthode au blanc de lessive à des personnes-clé dans les PNT de 85 pays.

RÉSULTATS : Dans 15 des 19 études trouvées, on a noté une amélioration statistiquement significative de la proportion de tests positifs (sensibilité) allant de 7% à 253%. La majorité (73%) des personnes avaient entendu parler antérieurement de la méthode au blanc de lessive. Quarante-quatre pour cent pensaient qu'elle pourrait améliorer la détection des cas dans leurs pays, alors que 49% l'ignoraient. Quatre-vingt-treize pour cent d'entre elles auraient donné la préférence à la méthode au blanc de lessive ; les raisons les plus courantes de le faire seraient des recommandations provenant de l'OMS ou de l'UICTMR ou encore des études positives réalisées dans leur propre pays. La méthode au blanc de lessive n'était utilisée en routine que dans trois pays seulement.

CONCLUSION : Les preuves sont suffisantes pour recommander d'évaluer et d'introduire la méthode au blanc de lessive dans la plupart des contextes où les cultures mycobactériennes ne sont pas effectuées en routine.

RESUMEN

CONTEXTO : Se ha sugerido que la sensibilidad del examen microscópico directo de la expectoración puede ser mejorada si el esputo es sometido a licuefacción con hipoclorito de sodio (NaOCl o cloro doméstico) y concentrado por centrifugación antes de la tinción para los bacilos ácido-alcohol resistentes.

OBJETIVO : Resumir los resultados de los estudios de cloración para mejorar la sensibilidad del examen microscópico de esputo y describir la opinión y los conocimientos de personas clave de los programas de control de la tuberculosis (PNT) con respecto a este método.

DISEÑO : Buscamos estudios que comparan el método de cloración con el examen microscópico directo del frotis de esputo en países de escasos o medianos recursos económicos, en Medline, EMBASE y Web of Science. Cada estudio fue evaluado con respecto a la metodología y al campo de aplicabilidad. También se enviaron cuestionarios concernientes al método de cloración a personas clave de los PNT de 85 países.

RESULTADOS : En 15 de los 19 estudios revisados había un mejoramiento estadísticamente significativo de la proporción de tests positivos o de la sensibilidad, que iba de 7% a 253%. La mayoría de las personas clave (73%) habían oído hablar del método anteriormente. El 44% de estas personas pensaban que este método podría mejorar la detección de casos en sus países, mientras que el 49% no sabía. El 93% de ellos promoverían el método de cloración ; las razones más frecuentemente evocadas serían las recomendaciones de la OMS o de la UICTER o estudios favorables realizados en los propios países. El método de cloración era usado de rutina sólo en tres países.

CONCLUSIÓN : Hay razones evidentes para recomendar la evaluación y la introducción del método de cloración en la mayoría de los sitios donde no se realiza rutinariamente el cultivo de micobacterias.