Frequency of isolated positive sputum cultures among pulmonary tuberculosis patients

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ABSTRACT

Objective: The occurrence of isolated positive sputum cultures among pulmonary tuberculosis (PTB) patients during treatment is not widely reported. This study describes the frequency of isolated positive sputum cultures among PTB patients after initiation of chemotherapy.

Methods: Fifty sputum culture positive PTB patients, consisting of 38 (76%) males and 12 (24%) females with a mean age of 34.31 ± 19.54 (13-75) years, were studied prospectively over a period of 10 months (September 1999 to June 2000) at Sahary Chest Hospital, Riyadh, Kingdom of Saudi Arabia. The majority of patients received standard 4-drug short-course antimicrobial chemotherapy. Weekly sputum smears and cultures were carried out for each patient during the treatment.

Results: Isolated positive sputum cultures were encountered in 4 (8%) of the studied patients. Two of them had 2 consecutive positive smears and cultures in their third and fourth week during treatment and were attributed to poor compliance to the treatment. Of the remaining 2 patients, one had single isolated culture in his fourth week, while the fourth patient showed a slight growth in the fifth week of the treatment. Due to the undetermined status of the smear and very few colonies in the culture, these 2 isolated cultures were attributed to the carry-over contamination during the laboratory procedures. The same treatment was continued with strict monitoring of compliance to the treatment and laboratory protocols. All of the 4 patients converted to negative before leaving the hospital.

Conclusion: Isolated positive sputum cultures might appear (infrequently) during treatment either due to the treatment noncompliance or carry over contamination. However, under either circumstance, the same treatment should be continued along with strict monitoring of treatment compliance and specimen decontamination and related laboratory protocols.

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Despite the availability of powerful and potentially effective treatment, tuberculosis (TB) remains an important cause of morbidity and mortality in many parts of the world.[1] There were an estimated 8.4 million new cases in the year 1999 and approximately 8 million in 1997, thus showing an increase of 100,000 new cases each year.[2-3] If the present trend continues, 10.2 million new cases are expected to be registered in the year 2005.[2-3] The management of TB during pregnancy and lactation is of special importance. Untreated TB cases pose serious threat to both mother and infant. Isoniazid (INH) prophylaxis is a serious consideration for infants born to mothers with active pulmonary TB. In the Kingdom of Saudi Arabia (KSA) where health care facilities are relatively better, TB is still common, particularly among expatriate workers from the Asian and Far-Eastern countries.[4]

The most simple and reliable way of establishing the diagnosis for pulmonary TB (PTB) is to find *Tubercle bacilli* in a dried smear of sputum by Ziehl-Neelsen (Z-N) staining or ultraviolet (UV)

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fluoroscopy with subsequent confirmation by sputum culture.[5] Once the treatment is initiated, bacteriologic evaluation is the preferred method of monitoring the treatment compliance as well as the therapeutic response. Patients are, therefore, subjected to extensive bacteriological follow-up during and after treatment through sputum cultures. Anti-TB therapy is continued until the sputum cultures are converted to negative, the radiological/clinical picture is satisfactorily improved, and the treatment course is completed.[6,7] A positive sputum culture is termed "isolated" when it is preceded and followed by several successive bacterial cultures, which fail to yield growth of *Mycobacterium* tuberculosis (MTB).[8-11] The occurrence and significance of such isolated positive cultures was first reported by Segawa et al,[8] and Lattimer et al[9] in the urine culture of patients with genitourinary TB. Later, Mitchison et al[10] and Aber et al[11] highlighted the origin of isolated positive sputum cultures among PTB patients. Since then, several studies have investigated the occurrence and clinical significance of isolated positive sputum cultures during or after chemotherapy.[12-16] The occurrence of such isolated positive sputum culture(s) during or after treatment may cause erroneous diagnosis, unnecessary therapy interventions as well as an extra burden on the public health system.[12-20]

The purpose of this prospective study was to estimate the frequency of isolated positive sputum culture(s) among PTB patients during the treatment, identify the source(s) and investigate the cause(s). In addition, the importance and need of negative sputum culture(s) at the time of discharging a PTB patient is also briefly addressed.

Methods. Patients attending the outpatient clinic at Sahary Chest Hospital, Riyadh, KSA, were recruited for this study during the year 1999-2000. Diagnosis of PTB was based on one or more of the following criteria: 1. signs and symptoms, such as persistent cough, hemoptysis, fever, night sweating or demonstration of acid-fast bacilli (AFB) in the Z-N stained sputum smear; 2. positive tuberculin skin testing (TST); 3. positive sputum culture; and 4. positive radiological findings. Tuberculin skin testing was performed strictly according to the World Health Organization protocol by injecting intradermally 0.1 ml of tuberculin solution (5 IU purified protein derivative) on the volar surface of the left arm. Indurations were recorded after 48 - 72 hours, and a size of ≥10 mm was considered positive.

For sputum culture, early morning sputum specimens, received in the microbiology laboratory, were decontaminated using 2% sodium hydroxide/N-acetyl-L-cysteine reagent and then processed for AFB smear and sputum culture according to the standard protocols. Ziehl-Neelsen stained smear were examined using 100 x magnification. A smear was reported "negative" when no AFB could be seen after viewing 300 fields; "indeterminate" (±) when only 1-2 AFB/300 fields could be seen; and "positive" when 1-9 AFB/100 fields or more were recorded. Patients with indeterminate (±) smears were requested to submit additional specimens, and the microscopic examination was repeated. Decontaminated sputum sediment (0.5 ml) was inoculated into BACTEC® (Becton Dickinson, Maryland, USA) 12 B liquid medium vials. The remaining decontaminated sputum specimen was mixed with 1 ml of 0.2% bovine serum albumin (BSA), and then inoculated onto Lowenstein-Jensen slants, which were incubated at 37°C in 5–10% CO_2 and examined weekly up to 8 weeks. BACTEC® 12 B vials were tested on a BACTEC® 460 TB system twice weekly for 2 weeks and once weekly thereafter for 4 weeks. Vials showing a growth index of ≥100 were tested for AFB by Z-N staining and only those patients who had the positive sputum culture were included in the study. Once the diagnosis of PTB was established, patients were hospitalized and the treatment was immediately started.

The demographic and clinical data of all the patients were carefully recorded. The majority of the patients were treated with standard 4-drug short term antituberculosis therapy including isoniazid (INH) 300 mg, rifampicin (RIF) 600 mg, streptomycin (STREPT) 1 gm, and pyrazinamide (PZA) 1.5 gms. However, STREPT was replaced by ethambutol (EMB) in patients with any renal problem or hearing deficiency. On the other hand, ethionamide (ETH) was withdrawn in patients with any report of optic neuritis or retinal changes. Patients reporting renal failure or optic neuritis were put on 3 drugs (INH-RIF-PZA) and both STREPT and ETH were stopped. All the medications were given once daily on empty stomach. After the initiation of chemotherapy, weekly sputum cultures of each patient were carried out to monitor the bacteriological conversion from positive to negative status. Two different technicians from the same laboratory recorded the results at different times on the same day.

Results. A total of 50 culture-proved PTB patients, consisting of 38 (76%) males and 12 (24%) females, with a mean age of 34.31 ± 19.54 (13-75) years, were studied prospectively. Distribution of patients according to various age groups is shown in **Table 1**. The most commonly encountered signs and symptoms among our patients were cough (94%), fever (70%), hemoptysis (44%), weight loss (36%) and dyspnea (10%). Duration of the symptoms ranged from 3 days to 2 years. Sputum smear was positive in 36 (72%) of the patients. Tuberculin skin

testing was positive in 43 (86%) patients, with 19 (38%) of them having an induration size of >20 mm.

Of the 50 studied patients, 34 (68%) were newly diagnosed cases, while 16 (32%) had previous history of PTB and received antituberculosis treatment in the past. Of these 16 known patients, one had amyloidosis induced nephrotic syndrome while 4 (25%) were resistant cases of PTB. The majority of the patients were treated with standard 4-drug, first-line antituberculosis therapy (**Table 2**). Three-drug (INH-RIF-PZA) therapy was prescribed to 3 (6%) patients who reported both renal impairment and optic neuritis. One resistant case with nephrotic syndrome was started with the second line of treatment consisting of INH, RIF, ETH and Cycloserine (regimen 4). Regimen-5 (INH-ETH-PZA-Thiacetazone) was given to only one patient who was a known and resistant case of PTB.

Table 3 shows the sputum conversion from positive to negative among our pulmonary TB patients. Of the 50 patients, one (2%) died of amyloidosis induced nephrotic syndrome during the first week of the treatment, while 4 (8%) never converted and left the hospital against the medical advice. **Table 3** shows that 97.7% of our patients, including 33 new and 11 known cases, converted to negative within 7-weeks of treatment.

Table 4 highlights the data of 4 patients who never converted to negative and left the hospital against medical advice. All of the 4 patients were male, with 3 of them having previous history of PTB, poor treatment compliance and drug resistance. All of them were smear and culture positive, and were started on the treatment regimens as indicated in **Table 4**. Despite monitoring of the treatment compliance, their sputum culture remained positive throughout their hospital stay. They refused any further treatment and left the hospital against medical advice after 28, 30, 49 and 84 days of hospital stay. All of those patients had positive sputum culture at the time of discharge, and none of them reported back in our outpatient clinics. The incidence of isolated positive sputum culture was reported in 4 (8%) of our PTB patients (**Table 5)**. Included among those were 3 men and one woman with a mean age of 27 ± 4.96 (20-31) years. All of them were PPD positive and had signs and symptoms indicative of active TB. First isolated positive sputum culture was seen in a 31-year-old female patient who did not had any past history of PTB. She was started on regimen-1 (INH-RIF-STREPT-PZA) and weekly sputum cultures were performed both on liquid and solid media. After showing 2 consecutive negative cultures in the 1st and 2nd week, an isolated positive culture appeared in the 3rd week with a growth index of 15 on BACTEC® System. In the 4th week, growth index decreased to <10, and finally it became negative in the 5th week. The AFB were identified (>6 organisms/100 fields) through Z-N staining of the direct smear prepared from the sputum and BACTEC® culture bottles. The same treatment (regimen-1) was continued under strict monitoring during this 2-weeks relapse period without altering the dose or drug. The culture became negative in the 5th week and stayed negative until the patient was discharged in the 9th week after showing 5 consecutive negative sputum cultures.

The second incidence of isolated positive sputum culture occurred in a relatively young, 20-year-old, male patient with a previous history of PTB and receiving antituberculosis treatment. He was started on INH-RIF-EMB-PZA (regimen-2) and weekly sputum smear and cultures were performed. His first isolated positive culture appeared in the 3rd week, after 2 consecutive negative smears and cultures in

Table 1 - Distribution of sputum culture positive pulmonary tuberculosis patients according to age groups.

Age groups (Years)	Patients n	(%)
Up to 20	6	(12)
21 - 30	21	(42)
31 - 40	9	(18)
41 - 50	9	(18)
>51	5	(10)
Total	**50**	**(100)**

Table 2 - Chemotherapeutic regimens prescribed to culture positive pulmonary tuberculosis patients.

Regimen	Drugs	Patients n	(%)
1	INH-RIF-STREP-PZA	33	(66)
2	INH-RIF-EMB-PZA	12	(24)
3	INH-RIF-PZA	3	(6)
4	INH-RIF-ETH-CYCLOSERINE	1	(2)
5	INH-ETH-PZA-THIACETAZONE	1	(2)

INH - isoniazid, RIF - rifampicin, STREPT - streptomycin, PZA - pyrazinamide, EMB - ethambutol, ETH - ethionamide

Table 3 - Sputum culture conversion among pulmonary tuberculosis patients.

Period of Conversion (week)	Patients converted n (%)	Regimen prescribed N of patients					Type of cases	
		1	2	3	4	5	New	Known
1st	15 (33. 3)	12	3	-	-	-	13	2
2nd	7 (15. 5)	5	1	1	-	-	5	2
3rd	8 (17. 8)	4	2	1	1	-	6	2
4th	4 (8. 9)	4	-	-	-	-	2	2
5th	3 (6. 7)	1	2	-	-	-	2	1
6th	6 (13. 3)	3	1	1	-	1	4	2
7th	1 (2. 2)	1	-	-	-	-	1	-
15th	1 (2. 2)	1	-	-	-	-	-	1
Total*	**45* (100)**	**31**	**9**	**3**	**1**	**1**	**33 (73.3%)**	**12 (26.7%)**

*One patient died during the 1st week of treatment. Four patients did not convert and left the hospital against medical advice.

Table 4 - Treatment outcome of patients who left hospital against medical advice.

Patient	Age (years)	Gender	Nationality	History	Treatment given	Duration	Treatment outcome
1	30	Male	Sudani	Known, resistant	INH-EMB-PZA THIACETAZONE	84-days	Left against medical advice with positive sputum
2	27	Male	Indian	Known, resistant	INH-RIF-EMB PZA	49-days	Left against medical advice with positive sputum
3	70	Male	Iraqi	Known, resistant	INH-RIF-EMB PZA	30-days	Left against medical advice with positive sputum
4	30	Male	Yemeni	Known, resistant	INH-RIF-STREPT PZA	28-days	Left against medical advice with positive sputum

INH - isoniazid, RIF - rifampicin, STREPT - streptomycin, PZA - pyrazinamide, EMB - ethambutol

Table 5 - The incidence of isolated positive sputum cultures among pulmonary tuberculosis patients.

Patient	Age (years)	Gender	Type of case	Treatment given	N of negative cultures prior to the isolated culture	N of isolated cultures (time of occurrence)	Treatment outcome
1	31	F	New (sensitive)	INH-RIF-STREP-PZA (Regimen 1)	2 (1st, 2nd week)	2 (3rd, 4th week)	Discharged after 5 consecutive negative cultures
2	20	M	Known (sensitive)	INH-RIF-EMB-PZA (Regimen 2)	2 (1st, 2nd week)	2 (3rd, 4th week)	Discharged after 5 consecutive negative cultures
3	27	M	New (sensitive)	INH-RIF-STREP-PZA (Regimen 1)	3 (1st- 3rd week)	1 (4th week)	Discharged after 3 consecutive negative cultures
4	30	M	New (sensitive)	INH-RIF-STREPT-PZA (Regimen 1)	4 (1st – 4th week)	1 (5th week)	Left against medical advice after 1 negative culture

INH - isoniazid, RIF - rifampicin, STREPT - streptomycin, PZA - pyrazinamide, EMB - ethambutol

the 1st and 2nd week of the treatment and remained positive until the 4th week. The AFB were identified in the direct smears prepared from the sputum and BACTEC® culture bottles. Regimen-2 was continued under monitoring and his culture became negative in the 5th week and stayed negative until his discharge after 5 consecutive negative sputum cultures. Third isolated culture was reported in a 27-year-old patient who had no previous history of TB, and was both smear and culture positive. He was started on INH-RIF-STREPT-PZA (regimen-1) and weekly smear and cultures were performed. Slight growth was observed in the 4th week of the treatment. The direct smear was negative, however, the smear prepared from the BACTEC culture bottle showed very few AFB (1-2 per 300 fields). The same treatment was continued and the culture became negative in the 4th week. The patient was finally discharged upon request after 3 consecutive negative cultures. Similar pattern was seen in another 30-year-old newly diagnosed male patient who was started on regimen-1. His BACTEC culture showed scanty growth in the 5th week of treatment, which proved to be mycobacteria on Z-N staining. However, his direct sputum smear prepared at the same time was negative. The patient was continued on the same treatment, his culture converted to negative in the 6th week, and he left the hospital against medical advice in the 7th week immediately after one negative culture.

Discussion. Affecting mainly the middle aged (20-40 years) population, TB has emerged as the world's worst infectious disease.[1-3] Sixty percent of our patients were between the age of 20-40 years, which is in agreement with earlier studies.[4,21,22] Previous reports from industrialized countries showed that TB mortality rate was higher in young adult women (15-44 years) than in men.[23-25] However among our patients, 76% were male and 24% female, which support earlier reports suggesting that men are more exposed to TB than women, being potentially more mobile than the female community.[21,22,26]

The diagnosis of active TB still largely depends upon the initial clinical suspicion and radiographic findings, followed by confirmation through bacteriologic investigations. In the vast majority of patients, primary infection is asymptomatic or minimally symptomatic, with fever, dry or productive cough, and occasionally, development of retrosternal pain or erythema nodosum.[27] Tuberculosis should be considered in the differential diagnosis of all patients with fever of unknown origin, night sweats, or unexplained weight loss. These symptoms usually develop soon after the onset of reactivation disease or secondary TB infection thereby prompting the patient to seek medical advice. In the present study, cough (94%), fever (70%), hemoptysis (44%), weight loss (36%) and dyspnea (10%), were the commonly encountered symptoms among our PTB patients. Our findings are supported by Dahmash et al[22] who also reported cough (85%), fever (60%) and weight loss (50%) as presenting symptoms among their TB patients. Our results are also in agreement with Korzeniewska-Kosela et al[25] who reported that the majority of their adult as well as elderly PTB patients had cough, weight loss and fever, followed by night sweats, and other nonrespiratory complaints.

A positive TST provides a simplest way to determine exposure to MTB, but does not necessarily indicate the existence of an active disease.[28-30] An induration size of 10 mm or more within 24-72 hours is usually considered significant.[31] Forty-three (86%) of our patients had a positive skin test with 19 (38%) of them having an induration size of >20 mm. Although microscopic identification of AFB on direct examination of Z-N stained sputum smear is good presumptive evidence of PTB, yet it does not exclude other mycobacteria diseases such as *Mycobacterium kansasii, Mycobacterium fortuitum* and others.[32] A definitive diagnosis requires identification of MTB in sputum culture. The outcome of the AFB smear testing is largely influenced by the quality of the sputum sample. An early morning sputum sample is likely to yield more conclusive results both in staining and culture as compared to the sample collected at the time of patient's visit to the clinic. That is why, in our opinion, 28% of our patients were smear negative. On the other hand, all of our 50 (100%) patients identified MTB in their sputum culture.

Short-course antituberculosis chemotherapy has been widely accepted and is desirable for at least all the smear-positive patients.[33-35] The broad consensus for short-course therapy is based on solid observations such as higher proportion of cured patients, less incidence of drug resistance, greater compliance with the treatment, and overall more cost-effectiveness.[33-37] We also used short-course chemotherapy in the present study, and 90% of our patients completed the prescribed course of the treatment with 97.7% of them converting to negative in less than 2 months (7-weeks) after the treatment started. Our results are in agreement with Singla et al[38] who have also reported a conversion rate of 96.7% among their pulmonary TB sensitive isolates after 3 months of therapy. One of our patients, with 10-year history of the disease, converted to negative in the 15th week after the treatment started. Being sensitive to INH-RIF-STREPT-PZA, the only probable cause of delayed response to the treatment was his uncooperative attitude as well as the history of being noncompliant to his treatment previously. It is

a well-known fact that even the most thorough and well-designed therapeutic regimen will fail without patient compliance.[37,39]

Four (8%) of our patients (both smear and culture positive), did not complete the therapy, and left the hospital against medical advice. Three of them were known case of PTB and had history of poor treatment compliance and multidrug-resistance. Recent studies have shown that young age, previous history of antituberculosis chemotherapy, and incorrect usage or poor treatment compliance are the risk factors for the development of drug-resistant MTB.[40-41] Those patients cannot be classified as "treatment failures" as they never completed their therapeutic course and defaulted against medical advice.

Despite strict monitoring of treatment compliance, their sputum culture remained positive throughout their hospital stay. Those patients refused any further treatment and left the hospital against medical advice after 28, 30, 49 and 84 days of hospital stay. All of those patients had positive sputum culture at the time of discharge, and none of them reported back in our outpatient clinic. Premature discharge of patients or discharge against medical advice is not uncommon among hospitalized patients.[26] Included in this category are usually the defaulters, patients transferred to other hospitals and those who died during the treatment. In our study, 4 patients with resistant PTB left the hospital against medical advice. Until now, there is no legal framework under which such patients could be detained in the hospital until the treatment is completed and they are fully cured with negative sputum. How far these patients carry the risk of causing infection to other members of the community is still controversial. Martin and Lazarus[27] compared the risk of infection to contacts of patients treated at home with those treated in a sanatorium and found no significant difference. They further added that the major risk of infection from an indexed case to patient's family or community is before the diagnosis is made and the treatment is started. Nash and Douglas[28] reported that patients with pulmonary TB transmit disease to others when he/she is not receiving adequate treatment, and the risk of causing infection to other members of the community is rapidly reduced when the patient starts receiving chemotherapy. Although, these studies offer reasonable evidence that risk of infection to the community is gradually reduced when a sputum positive PTB patient is receiving chemotherapy at home, yet the risk is still there, as the patient carry viable bacilli in her/his droplet nuclei. A PTB patient, undergoing antituberculosis therapy, should not be discharged until smear results become negative and treatment compliance is assured.

False positive or isolated positive cultures were reported in 4 (8%) of our patients. The occurrence of such isolated positive cultures among TB patients during chemotherapy is not an uncommon phenomena.[10,11,18,19] Mitchison et al[10] studied 37429 sputum specimens in the East African laboratories and reported the incidence of isolated positive culture in 405 (1.08%) specimens. In 2 separate studies, Burman et al[18] reported the occurrence of 4% false positive or isolated positive sputum culture among their PTB patients, and in a review of 14 studies he observed a median false positive rate of 3.1% (2.2-10.5%).[19] According to previous reports, an isolated positive culture could usually arise in 3 ways: 1. from the specimen of the patient; 2. by transfer of bacilli from positive to negative specimen in the laboratory; and 3. by clerical error.[10-11] The possibility of a clerical error seems to be comparatively rare, as the number of colonies reported in the isolated cultures was much less than reported in other positive cultures. Therefore, it is fair to conclude that isolated positive culture arose either from patient's specimen itself or from the laboratory transfer. Based upon the strict laboratory procedures for specimen handling adapted in our laboratory, the transfer of bacilli from positive to negative specimens during the procedure is of remote possibility, especially on 2 consecutive occasions. Therefore, it seems obvious that isolated positive cultures had apparently come from the sputum specimen of the patient. The occurrence of isolated positive culture suggests that either the patient has been noncompliant during that period or the organism is residual and persistently surviving despite chemotherapy. Moreover, it also appears that short course chemotherapy sometime is not capable of sterilizing the lesion even in patients who do not have true bacteriological relapse during extensive follow-up.[10]

In conclusion, our results indicate that the occurrence of isolated positive sputum culture in patients with PTB on treatment is not an unusual phenomenon, however, such an occurrence should alert the treating physician to patient's compliance with treatment or possible contamination during laboratory procedures or clerical error. Since the risk of infection caused by a TB patient to other members of the community is still controversial, it is, therefore, recommended that patients should be discharged from the hospital only when the sputum culture is converted to negative to ensure that the patient is not carrying viable bacilli in the sputum.

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