

ASSESSMENT OF THE MICROSCOPIC– OBSERVATION- DRUG - SUSCEPTIBILITY ASSAY (MODS) AND GENEXPERT ASSAY FOR DIAGNOSIS OF PULMONARY TUBERCULOSIS

By

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ABSTRACT

Background: Tuberculosis (TB) is considered the most common and highly contagious disease worldwide. Early and accurate diagnosis of TB with detection of multidrug-resistant Mycobacterium tuberculosis (M.TB) is of primary importance for both patient management and infection control.

Objectives: To evaluate the performance of Microscopic-Observation Drug-Susceptibility (MODS) assay and GeneXpert MTB/RIF(GeneXpert) assay in diagnosis of TB and detection of MDR-TB directly from sputum specimens in comparison to the conventional diagnostic methods (direct Ziehl-Neelsen "Z-N" stained smear and culture on Lowenstein-Jenson media "LJ").

Patients and Methods: After approval of institutional ethical committee a total of 120 suspected pulmonary TB patients admitted to chest hospitals and dispensaries in Egypt (Giza, Cairo, Alexandria, Dakahlia, Suez, Beheira, Monufia, Qena). Their age was more than 16 years; all of them did not receive any anti tubercular drugs. Sputum samples were collected from each patient and subjected to direct ZN smear, culture and sensitivity of M.TB on LJ and Middlebrook 7H9 medium for MODS assay. Finally Genexpert M.TB/RIF assay was done directly for all sputum samples.

Results: Infection rate among males was higher than female (81.7% & 18.7%). Also, it was observed that 56.7% of sputum samples gave positive direct Z-N smear, while 64.2% were positive for both LJ culture & MODS assay. On the other hand, by Genexpert, 68% of cases were positive. The sensitivity and specificity of the various diagnostic methods were studied in relation to LJ culture (gold standard). It was found that MODS assay was rapid that results were obtained in a median of 8 days ranged from (7– 21 days), while median time of LJ was 21days ranged from (21– 60). MODS had 100% sensitivity and specificity. Direct smear was less sensitive (88.3%), and GeneXpert was less specific (88.4%), but more rapid results were obtained in 2 hours. Finally, detection of MDR-TB using various methods for 77 isolated M.TB strains ranged from 16.9-19.5% to isoniazid (INH) and 9.8%-18.2% to rifampicin (RMP) by various diagnostic methods with statistically insignificant difference between them.

Conclusion: MODS was an optimal alternative rapid diagnostic technique for TB and detection of M.TB and MDR-TB in resource limited settings, results were obtained in a median of 8 days. On the other hand, Gene Xpert was a novel integrated diagnostic PCR analysis in a single hand free step for rapid diagnosis of TB and detection of RMP resistance in clinical specimens in 2 hours but it was expensive.

Key words: MODS, GeneXpert, M.TB, MDR-TB, TB, and PCR.

INTRODUCTION

Although much progress has been made in improving TB cure rates, diagnosis and case detection remain major obstacles to TB control. Conventional drug susceptibility testing (DST) methods for M. TB take approximately 6 weeks to yield results, which leads to loss of time and delayed initiation of proper treatment, resulting in the transmission of drug resistant strain in the community (*Pai et al., 2009*). Advances in technology have led to the development of newer modalities of DST based on automated systems or molecular diagnostic methods. These tests, however, are expensive. In resource-poor settings, a rapid, reliable yet inexpensive DST method is urgently required. The application of rapid, sensitive and inexpensive diagnostic tests such as the microscopic-observation drug-susceptibility MODS is capable of identifying MDR-TB aids in the early initiation of treatment and prevents further transmission (*WHO, 2017*). MODS assay is a phenotypic rapid diagnostic test for TB and testing for at least INH and RMP resistance (*Sanogo et al., 2017*). Rapid identification, which is essential for earlier treatment initiation, improved patient outcomes, and more effective public health interventions, relies on nucleic acid amplification techniques. The GeneXpert MTB/RIF assay is a novel integrated diagnostic device that performs sample processing and hemi nested real-time PCR analysis in a single hands-free step for the diagnosis of tuberculosis and rapid detection of RMP resistance in clinical specimens in two hours (*Wilson, 2014* and *Boehme & Nabeta, 2014*). The information provided by the GeneXpert aids in selecting treatment regimens and

reaching infection control decisions quickly (*Mohamed et al., 2019*).

PATIENTS AND METHODS

This work was conducted on 120 suspected patients of pulmonary tuberculosis. They were chosen according to the clinical manifestation and radiological data. These cases were selected from chest hospitals in Egypt and dispensary (Giza, Cairo, Alexandria, Dakahlia, Suez, Beheira, Monufia and Qena) from January 2016 to June 2019. Their ages were more than 16 years, (16 females and 104 males). All patients were complaining with prolonged night fever, fatigue, loss of weight, bloody sputum and positive radiological data. They did not receive any anti-tuberculous drugs. The collected sputum samples were processed in the central laboratories of Egypt.

Three sputum samples were collected from each patient in 50 ml. screw capped plastic sterile container. Each patient sample was subjected for direct Z-N smear, then culture and sensitivity of M.TB on L-J medium and Middlebrook 7H9 medium for MODS assay after sputum decontamination and processing. One ml of sputum sample was used for GeneXpert M.TB/RIF assay.

The following laboratory techniques were used :

- I. Smear staining by Z-N stain technique: was described by *Kent and Kubica (1985)*.
- II. Sputum decontamination and processing: Sputum decontamination is performed with the sodium hydroxide–N-Acetyl-L-Cysteine (NaOH-NALC)

method was described by *Kent and Kubica. (1985)*.

III. Culture and sensitivity of M.TB on L-J medium: Was done as Canetti methods (*Canetti et al., 1969*).

IV. Culture and sensitivity of M.TB on (MODS) assay: MODS assay was a liquid culture based test that detects M.TB and assessed (INH) and (RMP) susceptibility directly from sputum samples. This was done regarding the *MODS user guide (2008)*.

Twenty four well plates were inoculated with decontaminated sputum samples suspended in Middle brook 7H9 medium then examined for micro colonies which was detected in a medium after 7 days. A characteristic microscopic cording appeared of that growth in liquid media using an inverted light microscope. The growth was usually much earlier than macroscopic colony growth that can be seen on solid medium. The incorporation of isoniazid and rifampicin in the testing process enabled equally rapid MDR -TB detection. This method was characterized by the growth in liquid media is markedly faster than on solid media, an easily recognizable M.TB growth, and rapid detection of MDR strain of M.TB. This was done regarding the *MODS user guide (2008)*.

V-GeneXpert MTB/RIF assay: The GeneXpert Dx System integrates and automates sample processing, nucleic acid amplification, and detection of the target sequences in samples using real-time PCR (*Helb et al., 2010*).

Statistical analysis:

1. Coding of data was carried out manually, and analysis was conducted through SPSS program version 15 (*SPSS Inc., Chicago Ill, 2006*).
2. Descriptive and analytical statistical analyses were done.

Descriptive statistics:

- Quantitative data: mean and standard deviation “SD” were used as they measure central tendency and dispersion of quantitative data.
- Qualitative data were expressed in numbers and by using test of significance as: Chi square-test (χ^2): for comparison of percentage.

The study was reviewed and approved by the Research Ethics committee at the AL- Azhar University, Faculty of Medicine for Girls and by REC decision of the Ministry of Health and Population at the Central Directorate of Research and Health Development & Review.

RESULTS

A total of 120 suspected patients of pulmonary tuberculosis were selected and sent the collected sputum samples to Central Laboratories of Egypt from January 2016 to June 2019. Their ages were more than 16 years, (16 females and 104 males) (**Table 1**). It was demonstrated that 81.7% of patients were males (p value

< 0.001). According to geographic distribution, Giza, Cairo & Dakahlia proved more frequent rates of TB infection (30, 27.5, and 17.5, respectively).

Comparison between the various diagnostic tests of tuberculosis in relation to culture on L-J medium (**Figure 1**). We

found that the results of direct stained smears were the less than culture on L-J and MODS (56.7, 64.2, and 64.2), respectively. It was noticed that the results of MODS culture were as similar as L-J culture but it was more rapid (require 7-21 days incubation). On the other hand Gene Xpert gave higher results (68.3%), in which 82 sputum samples were positive. However five sputum samples which were negative using L-J medium and MODS culture proved positive results by Gene Xpert assay (**Figure 1**).

The sensitivity, specificity and accuracy of direct Z-N smear, MODS and Gene Xpert were studied in comparison to culture on LJ (gold standard). It can notice

the sensitivity, specificity & accuracy of MODS was 100%, while direct smear was less sensitive 88.3%. Also, GeneXpert showed 100%, sensitivity but specificity (88.4%) and accuracy 95.8% (**Table 2 and Figure 2**).

Moreover, the drug sensitivity test of M.TB isolates to INH using L-J medium and MODS assay (**Table 3**). The results showed a statistically insignificant difference between both methods (P value > 0.6). Also, drug sensitivity test of M.TB to RMP using culture on L-J, MODS and Gene Xpert (**Table 4**). The results diagnosed statistical insignificance difference between the various methods (P value > 0.4).

Table (1): Demographic data of 120 suspected tuberculous patients in relation to gender and geographical distribution.

Items	Suspected TB patients (n.=120)	
	n.	%
Age (years) - Range - Mean \pm SD	(>16 – 76) 45.3 \pm 14.3	
Sex - Male - Female	98 22	81.7 18.3
Residence -Giza	36	30.0
-Cairo	33	27.5
- Dakahlia	21	17.5
-A-Alexandria	16	13.4
- Beheira	7	5.8
- Qena	5	4.2
- Suez	1	0.8
- Monufia	1	0.8

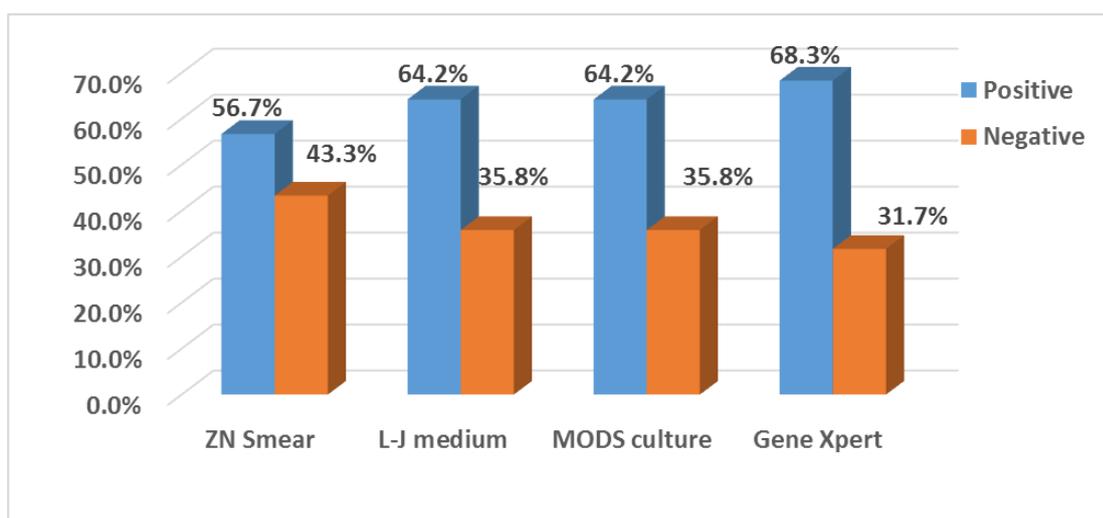


Figure (1): The results of four diagnostic tests used for 120 suspected pulmonary TB

Table (2): Comparison between the various diagnostic methods of tuberculosis in relation to culture on L-J medium (the gold standard)

Studied groups Methods	Culture on L-J medium				p.value
	Positive (77)		Negative (43)		
	No.	%	No.	%	
ZN Smear					
+Ve	68	88.3	0	0.0	0.001
-Ve	9	11.7	43	100.0	
Culture on MODS					
+Ve	77	100.0	0	0.0	0.001
-Ve	0	0.0	43	100.0	
Gene Xpert					
+Ve	77	100.0	5	11.6	0.001
-Ve	0	0.0	38	88.4	

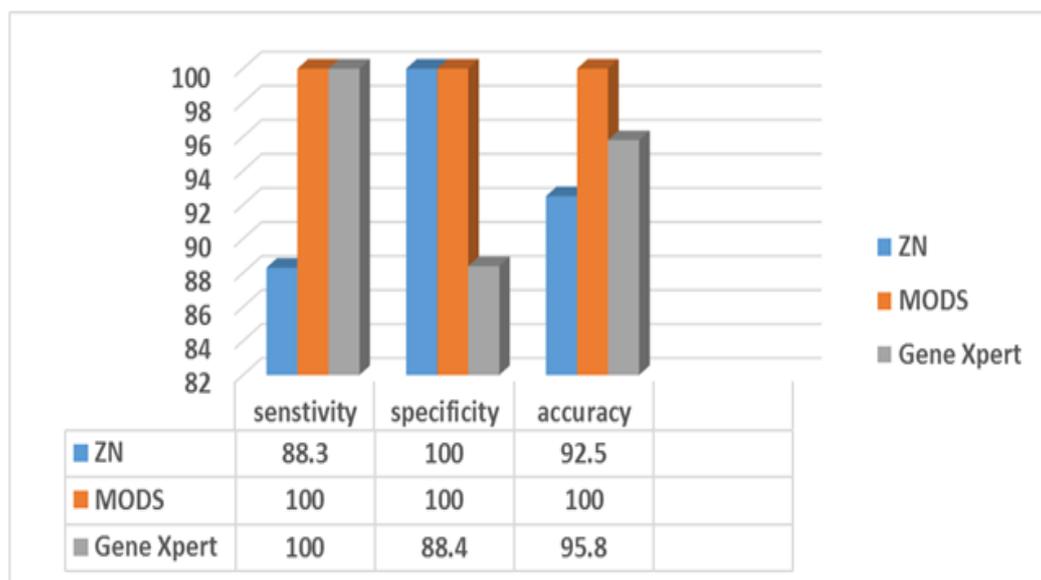


Figure (2): The sensitivity, specificity and accuracy of the various diagnostic methods of TB in relation to culture on L-J medium (the gold standard)

Table (3): Comparative study drug sensitivity test of 77 M.TB isolates to INH using L-J medium and MODS

Methods	INH		Resistant		p-value
	No.	%	No.	%	
Culture on L-J medium	64	83.1	13	16.9	0.676
Culture on MODS	62	80.5	15	19.5	

Table (4): Results drug sensitivity test of M.TB isolates to RMP using L-J medium, MODS and GeneXpert

Methods	RMP		Resistant		p-value
	No.	%	No.	%	
Culture on L-J medium	63	81.8	14	18.2	0.296
MODS assay	67	87.0	10	13.0	
Gene Xpert	74	90.2	8	9.8	

DISCUSSION

This work was carried out on 120 suspected patients of pulmonary tuberculosis. They were chosen according to the clinical manifestation and radiological data. The patients didn't receive anti-tuberculous drugs. These patients were selected from some chest

hospitals of Egypt and dispensary. It was observed that nearly three quarters of new cases, were males with statistically significant difference. These result was in agreement with the study done by *WHO (2018)* reported that males exceed females TB infection in Egypt due to socio-cultural factors. Men have more social

contacts and thus run a greater risk of exposure to contagious patient. Also exposure to predisposing factors like smoking, alcohol intake and drug abuse have considerable effects. More over immunological differences between men and women make males more susceptible than females to some infections (*Heba, 2014*).

In our study, comparison between the various diagnostic tests was presented. It was found the direct stained smear was the less sensitive than culture on L-J and MODS; however the result of GeneXpert gave higher percentage (68.3%).

It can be noticed that Z-N smear was cheap, rapid and widely used methods in all laboratories all over Egypt that with sensitivity (88.3%), specificity (100.0%), and accuracy (92.5%) compared with culture on L-J medium .

Our results were quite similar to that study done by *Husseiny et al. (2016)* from Egypt who reported that sensitivity (86.4%) and excellent specificity (99.7%) for Z-N. These results were also quite similar to those obtained by *Bunsow et al. (2014)* where Z-N showed sensitivity of (78.5%) and specificity of (98.3%) when compared with L-J culture as gold standard. In other study done by *Blakemore et al. (2010)* who compared acid fast bacilli (AFB) detection for diagnosis of TB by Z-N in relation to culture on L-J medium. It showed that (98%) sensitive and (48%) specific for smear-positive.

In this result, sensitivity of MODS was 100.0%, specificity 100.0%, and accuracy 100%, with median time 8 days for the detection of tubercle bacilli compared with the culture on L-J medium, while

median time of L-J was 21days. *Huang et al. (2015)* carried out a prospective study on MODS in 5 laboratories situated in China and found that sensitivity of MODS ranged between 87.78% and 94.4% and specificity between 96.8% and 100%.

Sanogo et al. (2017) found that the specificity and sensitivity of MODS assay to diagnose tuberculosis infection were determined in their population to be 88.73% and 88.24%, respectively; in positive sputum smears, naive to TB treatment. Also the result that obtained in Egypt by *Rasslan et al. (2012)* reported that, the sensitivity, specificity of MODS in the detection of M.TB were respectively 95.3% and 98.6%. MODS results were obtained in a median of 8 days while L-J were obtained in a median 21days.

The important of MODS elevated in most previous studies including ours because M.TB grows faster in liquid medium than in solid medium plus an inverted microscope is used to detect the cording, which is characteristic of M.TB in liquid medium but L-J culture detection of the colonies by naked eyes.

This was agreed with the study done by *Rasslan et al. (2012)* from Egypt they found that MODS assay is a rapid, economical, and highly sensitive and specific method for the detection of M.TB and DST directly from a sputum samples, results were obtained in a median of 8 days, but they were compared their result to BACTEC MGIT 960 and they were only examined known smear-positive patients, but in our study investigated a suspected TB patients and L-J was gold standard.

The current study revealed that the sensitivity of the GeneXpert compared with the culture on L-J positive specimens was (100.0%), specificity (88.4%), and accuracy (95.8 %). Near result was observed by *Mohamed, et al. (2019)* in Egypt who found that GeneXpert test has 97.6% sensitivity and 81% specificity, while *Iram et al. (2015)* from Pakistan, who reported 100% sensitivity and 86% specificity for Gene Xpert.

Husseiny et al. (2016) reported that the sensitivity and specificity of the GeneXpert assay were 93% and 98.3% respectively. *Monika et al. (2016)* found that the sensitivity and specificity of GeneXpert in sputum assay was (100%) and (90%). Other study of *Sharma et al. (2015)* who reported that sensitivity and specificity were 96.9% and 99.8% in this subject.

Our study found that GeneXpert detects 5 positive sputum samples which were –ve by L-J culture and MODS, Thus, the Gene Xpert assay has a sensitivity that approximately approaches that of culture. This is in agreement with *Bodmer and Ströhle,(2012)* who stated that the Gene Xpert overall clinical sensitivity of detecting M.TB in sputum of patients with suspected pulmonary TB when compared to L-J culture as the reference, this can be explained by death of many M.TB during processing and decontamination process of sputum samples while GeneXpert can detect both live and dead bacteria.

Finally, detection of MDR-TB using various methods for 77 isolated M.TB strains was ranged from 16.9%-19.5% to INH and 9.8% -18.2% to RMB by various diagnostic methods with statistically

insignificant difference between them (p value > 0.4).

This was similar to *WHO (2017)* who reported that MDR TB in Egypt increased that was reach 14% in 2016.

In comparison of various methods for diagnosis INH resistant, it was found that 19.5% (15) cases resistant and 2 cases mono resistant to INH on MODS while DST on L-J media detect 16.9% (13) cases, suggests that culture on MODS for diagnosis INH resistant was best for diagnoses. This in agree with *Rasslan et al. (2012)* found that the ability to detect MODS susceptibility for INH was more than RMP .

Sanogo et al. (2017) showed that MODS techniques showed identical mono-resistance to INH of 6.15%, this confirm that MODS more sensitive for detection of INH resistant more than RMP .

In our study, the comparison between the three tests in diagnoses of RMP resistant we noticed that in our result, L-J detect 14 cases resistant to RMP .

There were 6 cases only resistant to RMP on L-J media and 2 of them only resistant to RMP on MODS culture while GeneXpert detected 8 cases only it suggest that there is a mutation outside the M. TB rpoB gens (GeneXpert are specific only to rpoB gens) suggests that it may be resistant outside rpoB gene because Gene Xpert detect only 8 cases .

Sanchez (2015) found that GeneXpert was designed to identify RIF-resistance mutations in an 81-bp region (codons 426 to 452) of the rpo-β gene known as the RIF-resistance–determining region (RRDR).

The accuracy for identification of RMP resistance was 98 %. However, a study done in Swaziland demonstrated that the assay may not be able to detect wild type mutations for RIF-resistance outside the *rpo-β* I491F domain (*Lawn et al., 2013*).

This may clarify the difference in the results between the conventional and the GeneXpert method. In addition, this result can also be clarified by *Lawn and Nickol (2011)* who reported that to enable detection of rifampicin resistance by the Xpert, there must be present between 65 and 100% of the DNA from the RMP-resistant isolate depending on the mutation. They suggested that in patients with mixed infections, the GeneXpert assay might only detect the resistant strain if it was the predominant one present.

GeneXpert also has a limited ability to identify some RMP resistance. *WHO (2018)* recommended Cepheid GeneXpert® MTB/RIF Ultra cartridge (hereinafter Ultra MTB/RIF) that can both diagnose M.TB and indicate MDR- TB that more sensitive and specific.

CONCLUSION

MODS assay is a rapid, economical, and highly sensitive and specific method for the detection of M.TB and DST directly from a sputum sample and within 8 Days in comparable to L-J culture of M.TB and DST that take more time for its detection. Conventional DST takes more than 60 days to confirm the MDR in contrast to GeneXpert assay which is an extremely helpful diagnostic tool for the detection of rifampicin resistance in tuberculosis. Moreover GeneXpert assay is fairly highly sensitive and specific along with short turnout time in 2 hours but it is very expensive.

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تقييم طريقتي الفحص المجهرى والتفاعل الجينى التسلسلى في تشخيص الدرن الرئوى

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خلفية البحث: يعتبر السل الرئوى أكثر الأمراض المعدية شيوعاً في جميع أنحاء العالم.و يعتبر التشخيص المبكر والدقيق لمرض السل ومعرفة السلالات المقاومة للأدوية ذو أهمية أساسية لكل من المرضى ومكافحة العدوى.

الهدف من البحث: تقييم أداء اختبارين ذوي علاقة وهما المستتبت السائل مودز و التفاعل الجينى التسلسلى جين اكسبرت في تشخيص مرض السل الرئوى والكشف المبكر عن السلالات المقاومة للأدوية مباشرةً من عينات البصاق و المقارنة بطرق التشخيص الإعتيادية وهما صبغة الزيل نيلسين والمستتبت الصلب لوفنشتاين جينسين.

المرضى وطرق البحث: بعد موافقة اللجنة الاخلاقية بوزارة الصحة وكلية الطب بنات جامعة الازهر تم فحص 120 من مرضى السل الرئوى المشتبه بهم في مستشفيات ومستوصفات الصدر في مصر و كلهم لم يتلقوا أي أدوية مضادة للسل. تم جمع عينة البصاق من كل مريض والفحص المجهرى لها صبغة الزيل نيلسين مباشرة و الاستتبات والحساسية لجرثومة الدرن على لوفنشتاين جينسين و المستتبت السلل لفحص المودز وأخيرا تم إجراء فحص الجين اكسبرت لجميع عينات البصاق.

نتائج البحث: وجد أن معدل الإصابة بين الذكور كان أعلى من الإناث (81.7 % و 18.3 %) أيضاً قد لوحظ أن 56.7 % من عينات البصاق أعطت صبغة الزيل نيلسين إيجابية في حين أن 64.2 % كانت إيجابية لكلا لوفنشتاين جينسين و المودز. من ناحية أخرى، من خلال جين اكسبرت كانت 68 % من الحالات إيجابية. تمت دراسة حساسية وخصوصية طرق التشخيص المختلفة بالمقارنة مزرعة لوفنشتاين جينسين (المعيار الذهبى). وقد وجد أن فحص المودز كان سريعاً ، وكان له حساسية وخصوصية بنسبة 100% والفحص الميكروسكوبي

صبغة الزيل نيلسون كان أقل حساسية (88.3%) و جين اكسبرت أقل تحديداً (88.4%). أخيراً ، تراوحت عملية الكشف عن السلالات المقاومة للأدوية باستخدام طرق مختلفة لـ 77 سلالة السل الرئوى المعزولة تراوحت النسبة من 16.9% إلى 19.5% كانت مقاومة لعقار الازونيازيد وكذلك تراوحت النسبة من 9.8% - 18.2% كانت مقاومة لعقار الريفامبسين مع اختلاف ضئيل إحصائياً بينها.

الاستنتاج: طريقة مودز هي تقنية التشخيص السريع الأمثل لمرض السل الرئوى والكشف عن السلالات المقاومة للأدوية المتعددة في الدول محدودة الموارد و قد تم الحصول على النتائج في متوسط 8 أيام بينما لوفنيشتاين جينسين اعطت النتائج في متوسط ايام 21 من ناحية أخرى، يعد جين اكسبرت تحليلاً متكاملاً لتشخيص التفاعل الجينى التسلسلى في خطوة واحدة للتشخيص السريع لمرض السل والكشف عن السلالات المقاومة للريفامبسين خلال ساعتين ولكنه مكلف للغاية.