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ORIGINAL ARTICLE

BACTEC MGIT 960 SYSTEM IN THE SUSCEPTIBILITY TESTING OF MYCOBACTERIA TUBERCULOSIS TO THE FOUR FIRST-LINE ANTI-TUBERCULOUS DRUGS. COMPARISON WITH LOWENSTEIN JENSEN MEDIA

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Background: ***Tuberculosis (TB) is responsible for about one third of preventable deaths worldwide. A definitive diagnosis of mycobacterial infection depends on growth and identification of the bacteria. BACTEC 960 TB system is a state of the art, in-vitro diagnostic instrument designed and optimized for the rapid detection of mycobacteria from clinical specimens (except blood).***

Objectives: ***BACTEC 960 was evaluated for susceptibility testing to first line anti-tuberculous drugs, by comparing it with the standard method of proportion using Lowenstein Jensen media.***

Patients and Methods: ***Specimens were collected from 50 tuberculous cases. Culture and Susceptibility testing were performed at the microbiology laboratories of Abassia Chest Hospital, using BACTEC 960 and the proportion method using Lowenstein Jensen media.***

Results: ***The sensitivity of BACTEC 960 in detecting resistance to Streptomycin was 93.3% while the specificity was 77.1%, and the sensitivity of BACTEC 960 in detecting resistance to Isoniazid (INH) was 91.7% while the specificity was 97.4%. On the other hand, the sensitivity of BACTEC 960 in detecting resistance to Rifampicin was 61.5% while the specificity was 100%. The sensitivity of BACTEC 960 in detecting resistance to Ethambutol was 41.7% while the specificity was 92.1%. The time consumed for getting the results of the susceptibility testing using BACTEC 960 ranged from 6 to 15 days with a mean of 9.8 ± 2.48, while the time consumed for getting the results of the susceptibility testing using the proportion method ranged from 21 to 61 days with a mean of 38.28 ± 8.01and the paired difference between the two turnout times was statistically significant.***

Conclusion: ***The BACTEC 960 system is not yet sufficiently accurate to warrant the elimination of Lowenstein-Jensen media for susceptibility testing to first line anti-tuberculosis drugs.***

Keywords: ***Tuberculosis, BACTEC 960, rapid detection, drug susceptibility.***

INTRODUCTION

According to WHO Tuberculosis profile of Egypt published in 2011, 15 thousands incident cases, 23 thousands prevalent cases, 0.66 thousands deaths among HIV-negative people. Of the incident tuberculous cases; 0.055 thousands were HIV-positive. The total new and relapsed cases detected were 9260, including 4679 smear-positive TB, 1158 smear-negative TB, 21 unknown smears and 3048 extra-pulmonary tuberculosis. Retreated cases were 703. Among the notified new TB cases; 22% were multi-drug-resistant TB (MDR-TB), while among the notified retreatment TB cases; 38% were multi-drug-resistant TB (MDR-TB).[1]

A definitive diagnosis of mycobacterial infection depends on growth and identification of the bacteria.[2] To speed the bacterial culturing, time-consuming cultures on egg-based solid media, such as Lo¨wenstein-Jensen and Ogawa slants, are being replaced by faster liquid culture methods, such as the BACTEC MGIT 960 system (Becton-Dickinson, Sparks, MD) and the MB/BacT system (Organon Teknika, Boxtel, The Netherlands) and BACTEC 460.[3]

The BACTEC 460 system (Becton Dickinson, Sparks, Md.) has been marketed since 1977, however, this system requires radioactive reagents, causing waste problems, and vials have to be handled and punctured for readings at least eight times during 6 weeks of incubation, requiring a considerable amount of work and increasing the risk of cross-contamination.[4]

Multidrug-resistant tuberculosis (MDR-TB) is defined as TB caused by organisms that are resistant to isoniazid (INH) and rifampicin, the two first-line anti-tuberculous drugs. The emergence of extensively drug-resistant TB (XDR-TB), which is defined as MDR-TB that is resistant as well to any one of the fluoroquinolones and to at least one of three injectable second-line drugs (amikacin, capreomycin or kanamycin), has heightened this threat.[5] The most widely used method for Mycobacterium tuberculosis drug susceptibility testing is the proportion method, either on solid medium or on liquid broth.[6]

BACTEC 960 was applied in Egypt, only at Abassia Chest Hopspital in 2009; replacing the old radiometric BACTEC 460 system.

Aim of the work: Evaluation of BACTEC 960 for susceptibility testing to four first line anti-tuberculous drugs in comparison to the proportion method using Lowestein Jenesen media.

PATIENTS AND METHODS

The study was conducted on 50 tuberculosis cases diagnosed by positive culture. The collected specimens varied according to the anatomical site of the disease. Each patient was subjected to:

1. Full Clinical examination.
2. Chest radiography.
3. Tuberculin test
4. Specimen collection according to the anatomical site of the disease.
5. Bacteriological examination of specimens including:
   a) Direct smear stained with Ziehl-Neelsen stain.
   b) Culture using Lowenstein-Jensen media.
   c) Susceptibility testing to the four first- line anti-tuberculous drugs (Streptomycin, Isoniazid, Rifampicin & Ethambutol) was done using the Proportion Method using Lowenstein-Jensen media, which was the gold standard method. The agar proportion method allows for the quantitation of the proportion of organisms that is resistant to a given drug at a specified concentration. For a test to be valid, isolated, countable colonies (50 to 150) must be obtained on the drug-free medium. The number of colonies observed on the drug-containing medium is then compared with the number on the drug-free medium.[7]
   d) Culture using BACTEC MGIT 960.
   e) Susceptibility testing to the four first line anti-tuberculous drugs (Streptomycin, Isoniazid, Rifampicin & Ethambutol) was done using BACTEC MGIT 960: The automated BACTEC Mycobacterial Growth Indicator Tube (MGIT) 960 TB system is a state of the art, in-vitro diagnostic instrument designed and optimized for the rapid detection of mycobacteria from clinical specimens (except blood). This system has a 960-tube capacity for nearly 8000 specimens per year and is useful in laboratories dealing with large specimen loads.[8] For the performance of drug susceptibility test using BACTEC MGIT 960, drug-containing and drug-free control vials are inoculated with a standardized inoculums of the M. tuberculosis isolate and entered into the machine in a special rack-carrier with a printed barcode; this is read by the machine when entering the tubes to identify the test and apply the adequate algorithm for susceptibility or resistance interpretation. All readings are performed inside the machine and the results are printed as susceptible or resistant.[9] 5 MGIT tubes were labeled for each test culture; one for GC (growth control, without drug), one for SM

(S), one for INH (I), one for RIF (R), and one for EMB (E). Aseptically, 0.8 ml of BACTEC 960 SIRE Supplement was added to each of the MGIT tubes. Then 0.5 ml of the well-mixed culture suspension (inoculum) was aseptically added to each of the drug containing tubes using a pipette. Finally, labeled tubes were placed in the correct sequence in the set carrier (GC, SM, INH, RIF, EMB) and was entered into the BACTEC MGIT 960 instrument. The instrument printout indicated the susceptibility results for each drug. The instrument interpreted results at the time when the growth unit (GU) in growth control reaches 400. Results were either: (S) Susceptible, when the GU of the drug tube is less than 100, (R) Resistant if the GU of the drug tube is 100 or more and (X) Error when indeterminate resulted from certain conditions which may affect the test.

The performance characteristics were calculated for evaluation of BACTEC 960 as a method of susceptibility testing to the four anti-tuberculosis drugs, according to Laszlo et al.[10] & Goloubeva et al.[11] The agreement between the two methods was estimated by the Kappa statistic. The kappa value, a measure of test reliability, was interpreted as follows: < 0.2, poor; 0.21 to 0.4, fair; 0.41 to 0.6, moderate; 0.61 to 0.8, good; > 0.81, excellent.[12]

RESULTS

The study was conducted on 50 tuberculous cases at Abbassia Chest Hospital. Specimen type varied according to the anatomical site of the disease, as in Table 1.

Table 1. Numbers and percentages of different specimen types.

Type of specimen	Sputum	BAL	Pus	Pleural fluid aspirate	FNA
Number (**%**)	23 (46)	10 (20)	10 (20)	2 (4)	5 (10)

BAL: Broncho-alveolar lavage, **FNA:** fine needle aspiration.

Direct smear examination for AFB of the 50 specimens were positive in 50% of the cases and negative in 50%, as shown in Table 2.

Table 2. Classification according to direct smear examination of the specimens.

Direct smear	Positive	Negative
Number of cases	25	25
(%)	(50)	(50)

There was no difference between the results of both methods; all of the 50 specimen were culture-positive. The turnout time for obtaining a positive culture using BACTEC 960 ranged from 4 to 54 days with a mean of 13.22± 8.05, while the turn-out time for obtaining a positive culture using the Lowenstein Jensen media ranged from 21 to 64 days with a mean of 36.66 ± 12.18, and The paired difference between the two turnout times was highly statistically significant (p value <0.001).

The sensitivity of BACTEC 960 in detecting resistance to Streptomycin was 93.3% while the specificity was 77.1%. While the sensitivity of BACTEC 960 in detecting resistance to INH was 91.7% while the specificity was 97.4%. On the other hand, the sensitivity of BACTEC 960 in detecting resistance to Rifampicin was 61.5% while the specificity was 100%. And the sensitivity of BACTEC 960 in detecting resistance to Ethambutol was 41.7% while the specificity was 92.1%, as in Table 3.

Table 3. Sensitivity and specificity of BACTEC 960 for susceptibility testing to SM, INH, RIF and EMB.

	SM	INH	RIF	EMB
Sensitivity	93.3	91.7	61.5	41.7
Specificity	77.1	97.4	100	92.1
PVR	63.6	91.7	100	62.5
PVS	96.4	97.4	88.1	83.3

SM: streptomycin, **INH:** isoniazid, **RIF:** rifampicin, **EMB:** ethambutol, **PVR:** predictive value of resistance, **PVS:** predictive value of sensitivity.

By comparing the difference between BACTEC 960 and the proportion method in detecting drug resistance and sensitivity, and By conventional criteria, this difference is considered to be not statistically significant (pvalue>0.05), as shown in Table 4.

Table 4. Comparison between BACTEC 960 and the proportion method in detecting mono-resistance, poly-resistance and drug- sensitive tuberculosis.

	Mono-resistance	Poly-resistance	Sensitive	X2	P value
BACTEC 960	8	14	28	1.51	0.47
The proportion method	13	12	25		

The overall level of agreement (efficiency) between the BACTEC MGIT 960 susceptibility results and those of the proportion method was 89%. By comparing the susceptibility testing results using BACTEC 960 and the proportion method in case of streptomycin, there were 41 concordant and 9 discrepant results and Kappa value was 0.622. The strength of agreement was considered to be 'good'. While for INH 48 concordant and 2 discrepant results were found and Kappa value was 0.890. The strength of agreement was considered to be 'excellent'. As regards rifampicin, there were 45 concordant and 5 discrepant results and Kappa value was 0.703. The strength of agreement was considered to be 'good'. While for ethambutol there were 40 concordant and 10 discrepant results and Kappa value was 0.381. The strength of agreement was considered to be 'fair ', as shown in Table 5.

Table 5. Concordant & Discrepant susceptibility testing results of BACTEC 960 to the four anti-tuberculosis drugs in relation to susceptibility testing results of the Proportion Method.

Drug	Concordant results	Discrepant results	kappa statistic	Strength
SM	41	9	0.622	Good
INH	48	2	0.890	Excellent
RIF	45	5	0.703	Good
EMB	40	10	0.381	Fair

SM: streptomycin, **INH:** isoniazid, **RIF:** rifampicin, **EMB:** ethambutol.

By comparing the susceptibility results of both BACTEC MGIT 960 (the evaluated test) with the results of the proportion method using Lowenstein-Jensen media (the gold standard), there were 4 concordant and 4 discrepant results in detecting mono-drug resistance, while there were 11 concordant and 3 discrepant results in detecting poly-drug resistance. While there were 18 concordant results and 10 discrepant results in detecting drug sensitive tuberculosis. And as regards to muti-drug-resistance, there were 8 concordant and no discrepant results, as shown in Table 6.

Table 6. Concordant and discrepant result of both BACTEC 960 and the proportion method in detecting Mono-resistance, poly-resistance and drug-sensitive tuberculosis.

		BACTEC 960			
		Mono-resistance	Poly-resistance	Sensitive	Total
The proportion method	Mono-resistance	4	0	9	13
	Poly-resistance	0	11	1	12
	Sensitive	4	3	18	25
Total		8	14	28	50

The total efficiency of BACTEC 960 was 82% for susceptibility testing to streptomycin, while for INH it was 96%. While it was 90% for the susceptibility testing to Rifampicin and 80% in case of susceptibility testing to Ethambutol, as in Table 7.

Table 7. The efficiency of BACTEC 960 for susceptibility testing to the first line anti-tuberculosis drugs.

	SM	INH	RIF	EMB
Efficiency	0.82	0.96	0.9	0.8
(%)	(82)	(96)	(90)	(80)

SM: streptomycin, **INH:** isoniazid, **RIF:** rifampicin, **EMB:** ethambutol.

The time consumed for getting the results of the susceptibility testing using BACTEC 960 ranged from 6 to 15 days and a mean of 9.8 ± 2.48. While the time consumed for getting the results of the susceptibility testing using the proportion method ranged from 21 to 61 days and a mean of 38.28 ± 8.01, and The paired difference between the two turnout times was highly statistically significant (p value <0.001).

DISCUSSION

In this study, under the routine conditions of the clinical microbiology laboratory, BACTEC MGIT 960 was evaluated for susceptibility testing to the four first line anti-tuberculosis drugs by comparing its results with those of the proportion method using Lowenstein-Jensen media. The four drugs included Streptomycin, INH,

Rifampicin and Ethambutol.

The specimen collected from the 50 cases included in the study varied according to the anatomical site of the disease, 33 (66%) respiratory specimens (10 were BAL and 23 were sputum), 5 specimens were FNA from cervical lymph nodes, 2 specimens were pleural fluid aspirate, and 10 specimens were pus. While in the study of Lu et al.[13] the majority (78.1%) of specimens were from respiratory tract sources, including sputum, bronchial washings, tracheal aspirates, broncho-alveolar lavages, and trans-bronchial biopsies. This difference is partly due to the smaller number of cases included in our study, in addition that Abassia Chest Hospital is concerned with admission and treatment of pulmonary tuberculosis cases and limited cases of extra-pulmonary tuberculosis (tuberculous lymphadenitis, and tuberculous pleural effusion and empyema).

There was no discrepancy between the culture results of both BACTEC 960 and Lowenstein-Jensen media. All of the fifty examined specimens were culture positive; out of which 25 (50%) were smear positive and 25 smear negative. And this disagrees to what Chien et al.[14] found in their study, in which out of the 365 specimens tested, 124 (34.0%) were culture positive, of which 77 (62.1%) were smear-positive and 47 (37.9%) were smear-negative. Of the 124 isolates recovered, 94.4% (117/124) were recovered in BACTEC MGIT 960 and 75.8% (94/124) on Lowenstein-Jensen media. This difference might be explained by that only culture positive cases were included in our study to be able to perform the susceptibility testing which was the main aim of the study. There was only one case excluded from this study as it was both smear and culture negative.
The turnout time for obtaining a positive culture using BACTEC 960 ranged from 4 to 54 days with a mean of 13.22± 8.05, while the turn-out time for obtaining a positive culture using the Lowenstein-Jensen media ranged from 21 to 64 days with a mean of 36.66 ± 12.18. And this was close to what Chien et al (14) had found in their study, where the time of detection of M. tuberculosis ranged from 1 to 42 days with a mean of 11.1 days by BACTEC MGIT 960, and ranged from 17 to 56 days with a mean of 30.7 days by Lowenstein-Jensen media. While Tafaj et al[15] found in their study that the mean time to detection for Mycobacteria isolates on BACTEC MGIT 960 system was 12.7 days ± 8.43; while that on the Lowenstein-Jensen medium was 26.18 ±15.39. Also this coincides with Lee et al[16] whose study showed that the mean time for detection of M. tuberculosis was 11.6 days with MGIT 960, but disagrees with mean time of detection using Lowenstein-Jensen media as it was 20.1 days. The paired difference between the turnout time of both methods was statistically significant and this agrees with Tortoli et al.[17] who also found that the turnout time difference between the two methods was statistically significant.

Susceptibility testing to Streptomycin using BACTEC 960 showed 41 concordant and 9 discrepant results, when compared to the proportion method using Lowenstein-Jensen media (the gold standard). And the strength of agreement was considered to be 'good'. The concordant results included 14 true resistance and 27 true sensitivity while the discrepant results included 8 false resistance and one false sensitivity. The sensitivity of BACTEC 960 in detecting resistance to streptomycin was 93.3% while the specificity was 77.1%.

As for susceptibility testing to Ethambutol using BACTEC 960, there were 40 concordant and 10 discrepant results, when compared with the results of the proportion method. And the strength of agreement was considered to be 'fair '. The concordant results included 5 true resistance and 35 true sensitivity while the discrepant results included 3 false resistance and 7 false sensitivity.The sensitivity of BACTEC 960 in detecting resistance to Ethambutol was 41.7% while the specificity was 92.1%.

Comparing the susceptibility testing to INH using BACTEC 960 with the proportion method, 48 concordant and 2 discrepant results were found. The strength of agreement was considered to be 'excellent'. Out of the concordant results there was 11 true resistance and 37 true sensitivity, while out of the discrepant results there was one false resistance and one false sensitivity. The sensitivity of BACTEC 960 in detecting resistance to INH was 91.7% while the specificity was 97.4%.

Meanwhile, by comparing the susceptibility testing to Rifampicin using BACTEC 960 with the proportion method, there were 45 concordant and 5 discrepant results. The strength of agreement was considered to be 'good'.Out of the concordant results there was 8 true resistance and 37 true sensitivity, while out of the discrepant results there was 5 false sensitivity and no false resistance. And the sensitivity of BACTEC 960 in detecting resistance to Rifampicin was 61.5% while the specificity was 100%.

The total efficiency of BACTEC 960 was 82% for susceptibility testing to streptomycin, while for INH it was 96%, and it was 90% for the susceptibility testing to Rifampicin and 80% in case of susceptibility testing to Ethambutol.

We couldn't compare these results as the available studies done for evaluating BACTEC 960 for susceptibility testing of M. tuberculosis to the first line anti-tuberculosis drug, were based on the comparison between its results and the results of BACTEC 460. As the aim was to replace this BACTEC 460 which is a radiometric system based on the modified version of the proportion method that provides results within 5 to 6 days, with a significant time savings,[18] with the automated non-radiometric BACTEC 960. And due to an increasing concern about radioactivity

and its disposal, there is a growing tendency to eliminate radioactivite BACTEC 460 from diagnostic laboratories.[19] And for that reason it was replaced by BACTEC 960 in Abbassia Chest Hospital.

The time consumed for getting the results of the susceptibility testing using BACTEC 960 ranged from 6 to 15 days with a mean of 9.8 ± 2.48. While the time consumed for getting the results of the susceptibility testing using the proportion method ranged from 21 to 61 days with a mean of 38.28 ± 8.01. And this was close to what Abe et al.[20] have found in their study, where the mean turn-out time for susceptibility testing using BACTEC 960 was 7 days and that of the proportion method was 4 weeks. The paired difference between the two turnout times was statistically significant, by conventional criteria.

CONCLUSION

The BACTEC 960 system is not yet sufficiently accurate to warrant the elimination of Lowenstein-Jensen media for susceptibility testing to first line anti-tuberculosis drugs.

RECOMMENDATIONS

Antimicrobial susceptibility testing is critical in prescribing an effective drug regime for a tuberculosis patient, especially in areas where drug resistance incidence is high. In resource-poor settings, the success of treatment is threatened by multi-drug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis, which highlights the need for a rapid, simple, and cost-effective method of culture and susceptibility testing. That's why further studies are needed to evaluate the performance of BACTEC 960 for susceptibility testing to the first line anti-tuberculosis drugs to better evaluate their sensitivity & the predictive values of resistance. Evaluation of a greater number of strains could help to explain the cause of the discrepancies & to improve the results obtained in this study. International organizations, biochemical companies & others must develop arrangement to support low-income countries, with higher prevalence of tuberculosis, with new techniques such as BACTEC 960 as a way to overcome the major global danger of tuberculosis & multi-drug-resistant tuberculosis (MDR-TB). As currently, in Egypt, BACTEC 960 is only available in Abassia Chest Hospital's laboratory department.

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