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Extended Abstract

Euro Biotechnology 2018: BACTEC MGIT 960 system and classic Lowenstein-Jensen culture in the diagnosis and drug susceptibility of Mycobacterium tuberculosis from pulmonary specimens, at the Pasteur Institute of Algeria- Ferhat Djoudi- AlgeriaPasteur Institute of Algeria

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Abstract

Tuberculosis is a very old infectious disease. The etiological agent of this pathology, Mycobacterium tuberculosis, was discovered in the 19th century by Robert Koch. The tubercle bacilli infected the first hominids and co-evolved with theme. This infection usually begins with inhalation of contaminated droplets emitted by ill individuals, with a very low minimum infective dose, ranging from 1 to 10 bacilli. According to the World Health Organization (WHO), nearly one-third of the world's population is infected with tubercle bacilli. In 2015, 87% of new cases occurred in 30 countries with high TB burden. Six countries accounted for 60% of new cases: India, Indonesia, China, Nigeria, Pakistan and South Africa. The incidence of this disease has declined by an average of 1.5% per year since 2000 and the evolution of its diagnosis and treatment saved 49 million lives between 2000 and 2015. Control of the disease begins with the identification of M. tuberculosis and the development of detection tools, including X-rays and tuberculin test.

Aim of the Study

The aim of this study is to verify the contribution of BACTEC MGIT 960 in the diagnosis of pulmonary tuberculosis, compared to classic culture on L-J medium, at the Tuberculosis and Mycobacteria unit in Pasteur Institute of Algeria.

Material & Methods:

Setting & Ethical Considerations:

The laboratory of tuberculosis and mycobacteria at Pasteur Institute of Algiers (IPA) occupies a key place in the fight against tuberculosis in Algeria, in addition to being the national reference laboratory for the diagnosis and anti-tuberculosis drug testing, it is implicated in supervision, monitoring and reporting results across the entire network of national laboratories involved in the diagnosis of tuberculosis. It is also a supranational laboratory cooperating with WHO for the Africa region.

Oral consents were obtained from all patients prior to specimen's collection, and ethical considerations were taken into account during all steps of the study. The patient's data and results were maintained in secure database.

Materials and procedures

Three types of pulmonary samples were included in the study: expectoration, gastric tubing and bronchial aspiration. The collection of these samples was done in clean spittoons. Each sample sent to the laboratory was accompanied with information sheet of the patient. The samples were stored at + 4°C and Z-N staining were directly performed. Poly-microbial samples subjected to prior decontamination before they were cultured, and Petroff's decontamination technique was used, without neutralization. For each sample, 2 tubes of Lowenstein-Jensen were inoculated. Before inoculating the MGIT tubes, samples were processed using the BBLMycoPrep kit containing a mixture of N-acetyl-L-cysteine and 2% sodium hydroxide (NALC-NaOH), following the Kubica's Protocol.

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Positive tubes in BACTEC MGIT 960 were systematically inoculated on Lowenstein-Jensen media and Ziehl-Neelsen staining was performed for each tube. Finally, a rapid identification with immuno-chromatoghaphic TBC ID was applied.

Antibiogram on solid medium, proportions method:

The colonies (about 1 mg) are placed in a 50 ml flask containing glass beads, stirred for 10 min, in order to dissociate the colonies. From the initial suspension and dilution 10-2, two L-J tubes containing, each, one of the first line anti-tuberculosis drugs: Isoniazid (0.2 mg/ ml), streptomycin (4 mg/ml), rifampicin (40 mg/ml) and ethambutol (2 mg/ml), were inoculated. For each sample, two single L-J tubes (without antibiotics) were inoculated as controls. L-J containing specific media tubes (TCH, PNB and PAS), for confirmation of identification, were inoculated with the initial suspension. First verification of tubes, after incubation at 37°C, was done the 28th day, a second and final lecture at the 42nd day. After counting of colonies number on both types of tubes, a proportion ratio between the number of colonies with antibiotics and the number of colonies on the control tubes is expressed as a percentage. Below 1% "critical proportion", the strain is sensitive, above or equal to 1%, it was considered as resistant. MDRs isolates were submitted to a second antibiogram, against Ofloxacin and Kanamycin, to verify the existence of XDR isolates.

Antibiogram on liquid medium, BACTEC MGIT 960:

The first day of the positivity of a MGIT culture tube is considered as day "D-0". Inocula for this antibiogram should be prepared between "D-1" and "D-5". Five tubes were labeled for each isolate to be tested, a growth control tube and 4 tubes containing the antibiotics; streptomycin (1 µg/ml), Isoniazid (0.1 µg/ml), rifampicin (1 µg/ml), ethambutol (5 µg/ml). Reading is done automatically every 60 minutes, the sensitivity test is recorded between 5 to 12 days, and the result is obtained in the form of a printed report.

Statistical Analysis:

Statistical analysis was performed to calculate frequencies and significant differences, using one-way ANOVA test or Kruskall-Wallis, when appropriate, or by chi-square and Fisher's exact test respectively. Association between variable under consideration were evaluated using contingency tables, and all reported p-values were two-sided. $p < 0.05$ was considered significant.

Results & Discussion:

During the last decades there has been a significant upsurge in the incidence of TB infections. It is obvious that the increase of the favorable factors to this disease as well as the emergence of multi-drug resistant strains, are major health problems for the international community, whether, in developed or developing countries. This alarming situation has led to research and development of a more rapid and efficient means of detection and treatment, with the aim of reducing mortality rates. The race against this disease is justified, among other things, by the fact that it is the most deadly, in front of AIDS in 2014. Some studies have shown that the combination of the liquid and solid medium is an essential principle for the diagnosis of tuberculosis. Nevertheless, in most African laboratories, this combination is rarely available because of the high costs of liquid media. As a result, only the solid medium (L-J) is used for culture and susceptibility testing.

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