

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال الله تعالى

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صدق الله العظيم

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Dedication

I dedicate this research to:

My Kind Mother and to the Soul of My Father

My Dear Wife

My brothers and sisters

My friends and my colleagues

All who has ever taught me any thing

All Sudanese TB Patients

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Abstract

Tuberculosis is still one of the major health problems worldwide, with high mortality and morbidity. While third of the world population is infected with TB bacilli, the situation is getting worse by the rising emergence of drug resistant strains of *Mycobacterium tuberculosis*. Conventional method for drug susceptibility testing requires months before results can be reported. However, rapid methods such as phage assay have been developed and recorded as useful tool for quick diagnosis.

This study is descriptive cross-sectional laboratory based study which aimed to evaluate the usefulness of phage assay compared to proportional method and PCR in the diagnosis of MDR TB, to estimate the prevalence of MDR TB among tuberculosis patients and to detect the presence of *rpoB* gene among multidrug resistant isolates.

This study was conducted in Kassala State during the period from August 2009 to January 2012. Sputum specimens were collected from ninety acid fast bacilli consented patients (54 males and 36 females). Sputum specimens were processed for direct D29 and culture. All successful cultured isolates were subjected to biochemical tests for phenotypic characterization and further genotypic confirmation was made by amplification of IS 6110. For drug susceptibility testing, proportional method was adopted followed by both indirect D29 and amplification of *rpoB* gene.

The results showed that both males and females in different age groups were infected with TB and those between 21-50 are of the highest infection rate. 21 (23.3%) of the specimens were categorized as rifampicin resistant by direct D29 method, 75/90 (83.3%) of the specimens showed growth on LJ medium similar to MTB complex

colonies while 5/90 (3.3%) were identified as rapid growers. 60 out of the 75 slow growers (80%) were confirmed as MTB complex members depending on their biochemical characters (PNB, catalase and nitrate reduction). DST result for the 60 MTB isolates showed that 31/60 were drug resistant and that isoniazid compose for the highest percentage of resistance (20/31), followed by rifampicin (19/31) while MDR was detected in 18/60 of the isolates. All the 60 slow growers were confirmed as MTB by their positive IS 6110 results and 15/60 were rpoB positive. In conclusion, the study highlighted the high prevalence of MDR TB in Kassala State. Moreover D29 phage method in its first trial of application in Sudan revealed high sensitivity and specificity, which when combined to its major character of time saving (3 days compared to 70 days in DST) makes it a promising method for rapid uncostly diagnosis of MDR TB.

مرض السل لا يزال واحدا من المشاكل الصحية الكبرى في جميع أنحاء العالم، مع ارتفاع في معدل الوفيات. بينما يصاب ثلث سكان العالم بعصيات السل، يزداد الوضع سوءا بسبب ظهور سلالات المتفطرة السلية المقاومة للأدوية. الطريقة التقليدية لاختبار الحساسية للأدوية يتطلب شهورا قبل أن يتم التوصل للنتائج. وبالرغم من ذلك، فقد تم تطوير الطرق السريعة مثل فحص الفيروس اللاغم للبكتريا وسجلت بوصفها أداة مفيدة للتشخيص السريع.

هذه الدراسة الوصفية المقطعية على الأساس المخبري هدفت إلى تقدير مدى جدوى الفحص بالفيروس اللاغم للبكتريا مقابل الطريقة النسبية التقليدية، وطريقة PCR في تشخيص مرض السل متعدد المقاومة للأدوية، لتقدير مدى انتشار السل المقاوم للأدوية بين مرضى السل، والكشف عن وجود جينة rpoB بين العزلات متعددة المقاومة للأدوية. وقد أجريت هذه الدراسة في ولاية كسلا في الفترة من أغسطس 2009 إلى يناير 2012. جمعت عينات من البلغم موجبة لوجود عصيات السل العنصية على الحمض لعدد 90 مريض بعد اخذ موافقتهم (54 من الذكور والإناث 36). تم تجهيز عينات البلغم لإجراء فحص D29 المباشر والتزريع. وتم إخضاع جميع العزلات الناجحة لاختبارات كيميائية حيوية لتحديد الشكل المظهري وتم التأكيد وراثيا بتفاعلات البلمرة الجزيئية IS 6110. واعتمدت الطريقة النسبية لإجراء اختبارات الحساسية للدواء، تليها كل من D29 غير

المباشرة وتفاعل البلمرة التسلسلي للجين المقاوم rpoB. أظهرت النتائج كل من الذكور والإناث في الفئات العمرية المختلفة قد أصيبوا بمرض السل والذين تتراوح أعمارهم بين 21-50 هي من أعلى معدلات الإصابة. وبينت الدراسة أن 21 (23.3%) من العينات كان من نوع السل المقاوم لعقار الريفامبيسين بواسطة طريقة D29 المباشر. 75/90 (83.3%) من العينات صنف على حسب النمو في وسط LJ كمماثلة لمجمع مستعمرات MTB في حين تم تحديد 5/90 (3.3%)، كسريعة النمو (المتفطرة غير السلية). وتأكد من وجود 60 من 75 عينة بطيئة النمو (المتفطرة السلية) (80%) تنتمي لمجمع MTB اعتمادا على الصفات البيوكيميائية (PNB، الكتاليز، اختزال النترات). وأظهرت نتائج اختبار الحساسية لـ 60 MTB من العزلات أن 31/60 كانت مقاومة للأدوية وشكل الإيزونيازيد أعلى نسبة للمقاومة (20/31)، يليه الريفامبيسين (19/31) في حين تم الكشف عن السل متعددة المقاومة للأدوية لـ 18/60 من العزلات. كما تم التأكد من أن كل الـ 60 عينة من العزلات بطيئة

النمو MTB بواسطة نتائج ايجابية لفحص IS 6110 و 15/60 كانت إيجابية.لفحص rpoB .

في الختام، هذه الدراسة سلطت الضوء علي ارتفاع معدلات انتشار السل الم قاوم للأدوية في ولاية كسلا. وعلاوة على ذلك كشفت ان طريقة الفيروس اللاغم للبكتريا D29 في المرة الأولى من تطبيقه في السودان ذو حساسية عالية ونوعية ، بالإضافة للميزة الرئيسية وهي توفير الوقت (3 أيام م قابل 70 يوما ل DST) مما يجعل منها وسيلة تشخيصية سريعة غير مكلفة لمرض السل متعدد الم قاومة للأدوية.

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