

Evaluation of Resazurin Micro Titer Assay Method for Detection of Vancomycin Sensitivity for Staphylococcus Aureus

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ABSTRACT

Background:

Resazurin reduction method was used firstly in industrial microbiology, mainly in the field of milk and milk product. Resazurin is a blue dye that does not fluoresce but becomes pink and fluoresces as it is reduced to resorufin by oxidoreductase within live cells without any biological effects. This study was conducted to introduce the Resazurin micro titer plate method as alternative susceptibility test, in this study a 100 pure staphylococcus strains were examined against Vancomycin, the achieved results were compared with the gold standard disc diffusion susceptibility method.

Methods: In this study 100 Staphylococcus aureus strains collected from different infected area, were examined using Resazurin micro titer plate method, and Vancomycin antibiotics. The study was conducted in the research laboratory in the Omdurman Ahlia University, Khartoum state May 2017.

Results: This study showed statistically significant relationship between Resazurin micro titer plate method and disc diffusion susceptibility method with P. Value of 0.03.

Conclusion: Resazurin micro titer assay method was compared to disc diffusion susceptibility method and was achieved a predictive value of 96% and it's may considered a valid susceptibility method.

Recommendation: Resazurin micro titer may introduce as a cheap antimicrobial susceptibility, furthermore; it will be a promising method and superior the old disc diffusion method because by it the MIC and MBC can be achieved. A large sample size is recommended.

Keywords: Resazurin Micro Titer Assay.

I. INTRODUCTION

Resazurin reduction method was used firstly in industrial microbiology, mainly in the field of milk and milk product ⁽¹⁾. Resazurin is a blue dye that does not fluoresce but becomes pink and fluoresces as it is reduced to resorufin by oxidoreductase within live cells without any biological affects ⁽¹⁾. This method produced the

most reliable and accurate results for bacterial and yeast contamination in milk ⁽¹⁾, and it is also has been used for rapid detection of the antibiogram against many antibiotics ⁽⁷⁾, especially with the first and second line of M. tuberculosis antibiotic ⁽¹⁻⁷⁾. Disc diffusion method remains the gold standard method for detecting antibiogram to verify bacterial types worldwide ⁽⁴⁾. Many disadvantages in disc

diffusion method; dryness of plate surface, thickness of medium, concentration of the agar and type of media as well as inoculums ⁽⁸⁾.

Resazurin micro titer assay methods is a simple method require neither fancy equipment nor expensive substrates or reagents, depending on reduction of Resazurin solution by alive bacterial action which revealed as color change in micro titer plate ⁽²⁾. (Redaction of Resazurin to resorufin indicate bacterial viability within antibiotics indicating that no bacteriostatic or bactericidal activity). Many previous studies were conducted to introduce the Resazurin reduction method as alternative susceptibility test; all these studies were focus on anti-tuberculosis antibiogram. Study that was done in UK by Ray and Mitch son concerning the role of sensitivity tests in short-term chemotherapy, using Resazurin micro titer assay method in detecting the anti-tuberculosis first line antibiotics ⁽¹⁾.

II. MATERIALS AND METHODS

Study design: In this study, 100 Staphylococcus aureus strains collected from different infected area, from infected patients, aged between (10-36) years at Omdurman Teaching Hospital, Khartoum during February to May 2017. The data was collected from hospital microbiology laboratory registration books. Ethical approval was taken from Al Neelain University Research Ethical Committee.

Experimental work

Different strains of Staphylococcus aureus isolated from infected patients and tested for Vancomycin sensitivity by using Resazurin Micro Titer Assay Method at microbiology laboratory – Omdurman Ahlia University.

Collection of specimens and processing

The working solution of 0.02% Resazurin, Vancomycin 4µg/ml was prepared as manufactured structure and Nutrient Broth medium was aliquot into a sterile tube. The inoculums was prepared from fresh bacterial growth on Nutrient agar slop medium, a confluent portion of the bacterial growth was swept out with a sterile cotton swab, and transfers into sterile tube contain NaCl and homogenized, strain stock solution was adjusted with sterile NaCl-solution to be equivalent to a 0.5 McFarland standard (1.5 x 10⁸ CFU/ml), ⁽⁶⁾.

The sensitivity test was carried out, briefly; 200 µl distilled water was added to the outer wells (A1 – A11), 100 µl of nutrient broth medium was added to each wells of B to G 2 – 11, then a 100 µl from inoculums and 100 µl from antibiotic working solution was add to each wells containing nutrient broth medium, positive and negative controls were added to B 10 and B 11 respectively, after that the plate was sealed by an sterile nylon cover and incubated aerobically at 37°C for an over 10 hours. After completing the incubation a 30 µl of 0.02% Resazurin solution was added to each wells, then the plate was sealed again with sterile nylon tape and incubated for farther 3 hours at 37°C.

Calculation and interpretation of results

The results were then record and interprets; colour change from blue to pink indicated bacterial growth and interprets as resistant to the Vancomycin antibiotic, no changes in the result was consider sensitive, indicating the bacterial death.

Data analysis

The generated data were analyzed by using Statistical Package for Social Sciences (SPSS) program.

III. RESULTS & DISCUSSION

A total of 100 *Staphylococcus aureus* strains isolated from infected patients, aged between (10-36) years old with mean 26.6 years old their mean age ranged in between (10-36) years 62 (62%) of them were females, 30 (30%) were males and 8 (8%) of them were children ,as shown in table 1.

The sensitivity and specificity of the Resazurin micro titer assay method were 96% and 4% respectively when compared to the disc diffusion method reproducing P – Value of 0.03, as shown in Table 2.

This finding is quite similar to that done by Hafez and his colleagues who studied 100 isolates of *S. aureus* for Cefoxitin and reported that there was a highly significant association between the results of the Cefoxitin (30ug) disc diffusion method and the results of the REMA ($P < 0.01$) (Table 2). The finding of this study is in agreement with the results of (3), who reported 100% agreement between the Resazurin micro plate assay and the broth micro dilution method for the detection of Oxacillin resistance in *S. aureus*. Moreover it reported 100% agreement between the Resazurin micro plate assay for the rapid determination of MRSA and the Cefoxitin MIC determined by the reference broth micro dilution method (3, 5).

Table 1. Distribution of study population according to the gender and mean of age

Participants	Frequency	Percentage	Mean/ years
Females	62.0	62.0%%	33.3
Males	30.0	30.0%%	35.5
Children	8.0	8.0%	10.9
Total	100	100.0%	26.6

Table 2. The Disc Diffusion Method, Resazurin micro-titter assay Cross-

		Count			Sensitivity	Specificity	P. value
		Disc Diffusion for					
		Sensitive	Resistant				
Resazurin micro-titter assay	Sensitive	96.0	0.0	100%	96.0%	0.03	
	Resistant	0.0	4.0%				

IV. REFERENCES

[1]. OBrien J, Wilson I, Orton T, Pognan F (2000). Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *Eur J Biochem*; 267: 5421-6.
 [2]. Anoopkumar D, Carey S, Conere JB, Allshire A (2005). Resazurin assay of radiation response in

cultured cells". *British Journal of Radiology* 78 (934): 945–7. doi:10.1259/bjr/54004230.
 [3]. Baker, C.N. and Tenover, F.C. 1996. Evaluation of alamar colorimetric broth microdilution susceptibility testing method for *Staphylococci* and *Enterococci*. *J. Clin. Microbiol.*, 34: 2654-59.
 [4]. Bauer AW, Kirby WMM, Sherris JC, Turck M. (1966). Antibiotic susceptibility testing by a

- standardized single disc method. Am. J. Clin. Pathol. 45:493–496.
- [5]. Hafez1, H. M. Abd El Hamid D. H , Tarek, D. and Goma F.A (2017) Resazurin Microplate Assay: Rapid Assay for Detection of Methicillin Resistant Staphylococcus aureus Int.J.Curr.Microbiol.App.Sci (2017) 6(4): 174-181
- [6]. Joseph Mcfarland ,M.D jama. 1907; xlix(14): 1176-1178.
- [7]. Lemus D, Martin A, Montoro E, Portaels F, Palomino JC. (2004) Rapid alternative methods for detection of rifampicin resistance in Mycobacterium tuberculosis. J Antimicrob Chemother; 54: 130-3.
- [8]. Martin A, Palomino JC, Portaels F. (2005) Rapid detection of ofloxacin resistance using two low-cost colorimetric methods: resazurin and nitrate reductase assays. J Clin Microbiol; 43: 1612-6.