Double Combination of Some Antibiotics Against Local Isolate of Methicillin Resistant *Staphylococcus aureus* (MRSA)*

Habeeb, S. N. and Rabab, J. H.
Department of microbiology, College of medicine, Univ. Babylon

Abstract:
We compared in this study the *in vitro* potency of some antibiotics when used singly and in combination against the multi-drugs resistant *Staphylococcus aureus* using the wells diffusion in agar. Rifampin, vancomycin, tetracycline and trimethoprim-sulfamethoxazole were randomly selected for this study. The isolate of *S. aureus* used in this study was previously characterized in our laboratory as methicillin-resistance *Staphylococcus aureus*-MRSA carrying the *mec-A* gene which is responsible for methicillin resistance.

Tremendous potencies were observed when the antibiotics used in combination compared with each antibiotic alone. The inhibition zone of vancomycin plus rifampin was 28 mm. in diameter compared with 18 mm. and zero (fully resistance) for vancomycin and rifampin respectively. Like rifampin, trimethoprim-sulfamethoxazole was inactive per se, since no inhibition zone was seen but when combined together, the inhibitory action significantly increased up to 19 mm. in diameter. Furthermore, combination of tetracycline with trimethoprim-sulfamethoxazole scored an inhibition zone as high as 43 mm. compared with 30 mm. for tetracycline alone (data for the later combination are not shown in this paper)*****.

*****This paper is a part of M.Sc. thesis, College of medicine, University of Babylon. The comprehensive data are included in the thesis (in press).

Introduction:

The first methicillin-resistant *Staphylococcus aureus* (MRSA) case was reported in United Kingdom in 1961, shortly after methicillin was introduced into clinical practice (1). Seven years later, MRSA became widespread in Japan, Europe, United Kingdom, Australia and United States (2). In Iraq, MRSA has been found to be the major nosocomial pathogen in different medical centers in Najaf province (3) and in AL-Diwaniya city as well (4). To overcome this clinical problem, vancomycin was recommended to treat the MRSA infections, but shortly later, vancomycin resistant *Staphylococcus aureus* (VRSA) emerged (5) and despite being considered the drug of choice for serious MRSA infections, vancomycin lost its activity and became inactive. Recently, in 2009, it has been recommended that, accurately combination of antibiotics with each other may effectively treat the serious infections of multi-drugs resistant MRSA (6).

The reasons for the use of antibiotics combination for the treatment of serious MRSA infections can be attributed to the following: to broaden coverage to include heteroresistant MRSA, to prevent the emergence of reduced susceptibility to antibiotics, to achieve bactericidal synergy, to penetrate cells and tissues not reached by single antibiotics, to provide activity against organisms growing in biofilm, to treat the polymicrobial infections and to inhibit toxin production (7). This is not always correct, that when two antimicrobial agents are combined, they may have one of the three effects *in vitro* against the organism: additive (indifferent), synergy or antagonism (8). The data presented in this paper are a part of M.Sc. thesis being suggested and designed to demonstrate the effects of antibiotics combination against local isolate of MRSA, which was previously isolated and fully characterized in our laboratory (4).

Materials and methods:

Bacterial isolate: A local isolate being isolated from sever burn infection and fully identified and characterized as multi-drugs resistant MRSA carrying the *mec-A* gene was kindly provided by AL-Fa'ady (College of medicine, Babylon University). The isolate was re-identified according to the routine methods to confirm the stability of its diagnostic characteristics.

Antimicrobial susceptibility testing: Muller-Hinton agar (Oxoid) was used for detection the antibiotics susceptibility by wells diffusion test. The following antibiotics were used with known potency (Himedia): vancomycin, rifampin, tetracycline, and trimethoprim-sulamethoxazole. The results of antibiotics susceptibility were interpreted according to CLSI, 2010, (9).

Results and discussion:

Figure-1 shows the result of combination of vancomycin with rifampin. Rifampin alone was completely inactive since the inhibition zone was zero. In contrast, vancomycin exhibited an inhibition zone which accounted for 18 mm. in diameter,

while the inhibition zone significantly increased up to 28 mm. in diameter when these antibiotics were combined together, which indicates for synergistic effect of these antibiotics against MRSA.

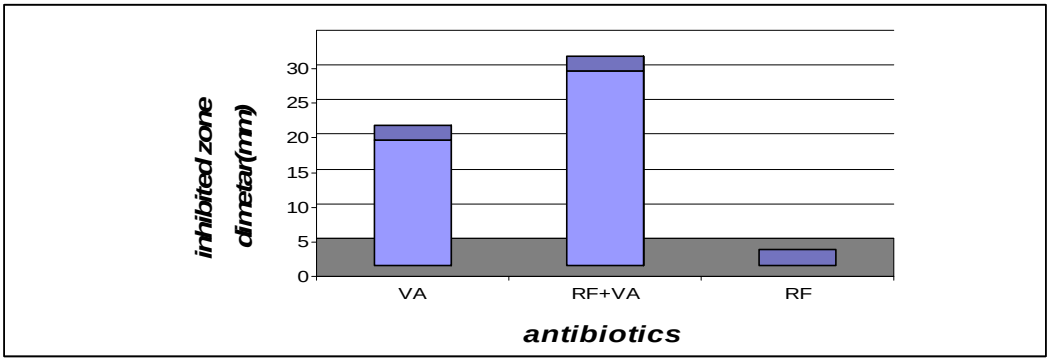

Figure-1: The effect of vancomycin and rifampin, each alone and in combination against MRSA.

This result strongly confirmed the result obtained by Deresinski, 2009 (7) who stated that rifampin was found to enhance the activity of vancomycin against MRSA. This enhancement has been also reported elsewhere (8).

Both, rifampin and trimethoprim-sulfamethoxazole were inactive, since no inhibition zone were seen for each as shown in fgure-2, but the inhibition zone went up to 19 mm. in diameter when these antibiotics were combined together. The effect of these antibiotics against MRSA is seemed to be synergy. This can be attributed in part to rifampin, which has number of characteristics that make it potentially effective when used in combination with other antibiotics, including its potent bactericidal activity, modest activity against non-growing cells and ability to penetrate cells (10).

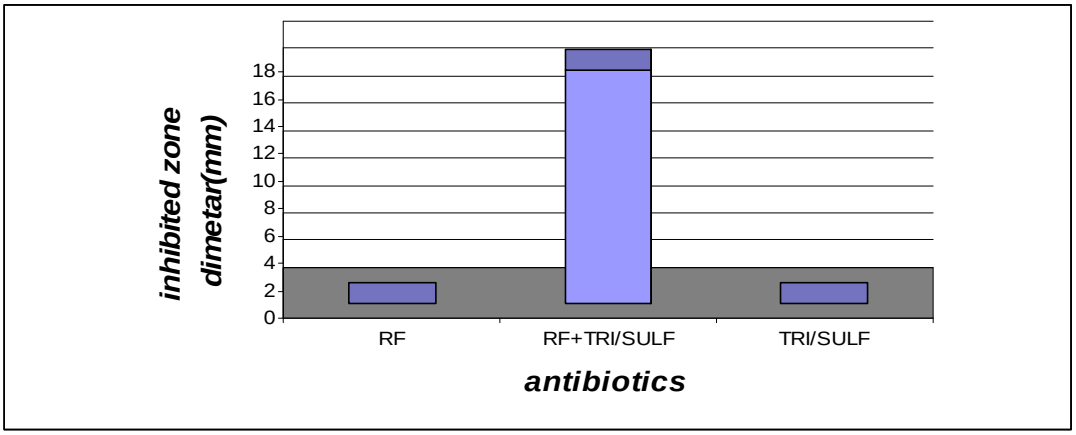

Figure-2: The effect of rifampin and trimethoprim-sulfamethoxazole, singly and in combination against MRSA.

In conclusion, in despite of successful findings being reported in this study, antagonism of antibiotics was also observed (data are not shown), which represents

the main disadvantage of antimicrobial combinations, but it still takes advantages of different mechanisms of action and/or toxicities profiles as indicated above. However, advantages and disadvantages are closely related with the selection of agents which should be dependent in part upon the type of antibiotic, the nature of action, the susceptibility patterns, the site of infection, and the type of microbe.

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References:

1- Baggett, H. C., Hennessy, T. W., Leman, R., Hamlin, C., Bruden, D., Reasonover, A., Martinez, P., and Butler, J. C.(2003). An outbreak of community-onset methicillin-resistant *Staphylococcos aureus* skin infections in south-westren Alaska. Infect. Hosp. Epidemiol. 24:397-402.

2- Barrett, F. F., McGehee, R. F., Jr.., and Finland,M.(1968). Methicillin-resistant *Staphylococcus aureus* at Boston City hospital. Bacteriological and epidemiological observations. N.Engl.J.Med.279:441-448.

3- Al-Sahllawi, Z., S., R. (2002). A bacteriological study on local isolates of methicillin resistant *Staphylococcus aureus*. M. Sc. thesis, College of science, Univ. Kufa.

4- Al-Fu'ady, A., H. (2010). Phenotypic and genotypic (*mec-A*) of methicillin-resistant *Staphylococcus aureus* isolates in AL-Dewaniya. M.Sc. thesis. College of medicine. University of Babylon.

5- Lodise, T.P., Graves, J. and Evanse, A. (2008). Relationship between vancomycin MIC and failure among patients with methicillin-resistant *Staphylococcus aureus* bacteremia treated with vancomycin. Antimicrob. Agents Chemothe. 52:3315-3320.

6- Baldoni, D., Haschke, M., Rajacic, Z., Zimmerli, W. and Trampuz, A. (2009). Linezolid alone or combined with rifapin against methicillin-resistant *Staphylococcus aureus* in experimental foreign-body infection. Antimicrob. Agents Chemothe. 3:1142-1148.

7- Deresinski, S. (2009). Vancomycin in combination with other antibiotics for the treatment of serious methicillin-resistant *Staphylococcus aureus* infections. CID. 49: 1072-1079.

8- Rose, W. E. and Poppens, P. T. (2008). Impact of biofilm on the *in vitro* activity of vancomycin alone and in combination with tigecycline and rifampicin against *Staphylococcus aureus*. J. Antimicrob. Chemother. 63: 485-488.

9- Clinical and Laboratory Standards Institute(CLSI), (2010). Performance standards for antimicrobial susceptibility testing. Approved standard M100-S17. Vol.27, No.1. NCCLS, Wayne, PA. USA.

10- Darouiche, R. O. and Hamill, R. J.(1994).Antibiotic penetration and bactericidal activity within endothelial cells. Antimicrob. Agent Chemother. 38: 1059-1064.