

Original Article

Double-disk synergy test for detection of synergistic effect between antibiotics against nosocomial strains of *staphylococcus aureus*

Rasool Soltani¹, Hossein Khalili², Fateme Shafiee¹

¹Department of Clinical Pharmacy and Pharmacy Practice, Isfahan University of Medical Sciences, Isfahan, Iran

²Department of Clinical Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

Objective: Synergistic effect between commonly used antibiotics against nosocomial multidrug-resistant strains of *Staphylococcus aureus*, if present, could provide a viable option as an alternative therapy for infections due to this pathogen. The aim of this study was searching for any synergistic effect between several antibiotics against drug-resistant strains of *S. aureus* with nosocomial origin using double-disk synergy test and to determine the applicability of this test for such a purpose.

Methods: Over a 6-month period, strains of *S. aureus* isolated from clinical specimens of hospitalized patients with documented nosocomial infection underwent disk diffusion test using antibiotic disks of oxacillin, cephalothin, clindamycin, ciprofloxacin, vancomycin, cotrimoxazole, rifampin, erythromycin, gentamicin and meropenem. Double-disk synergy test was performed for all isolates resistant to at least two of applied antibiotics. Combinations of all possible pairs of antibiotics (to which the microorganism was resistant) were tested by placing antibiotic disks at distance of 20 mm from each other (center to center). After 16-20 hours of incubation, if synergistic effect was present among two antibiotics, an inhibition zone was formed between their disks.

Findings: Among all of possible two-antibiotic combinations tested for 41 resistant isolates, only two cases of synergistic effect were detected; both effects were among rifampin and cotrimoxazole.

Conclusion: The combination of rifampin and cotrimoxazole could provide a viable option for treatment of infections due to resistant strains of *S. aureus*; however, clinical trials are needed before any new recommendation. Also, double-disk synergy test seems to be capable of detecting the synergistic effect between antibiotics at *in vitro* level.

Keywords: Antibiotic; double-disk test; *staphylococcus aureus*; synergism

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Corresponding author:
Dr. Hossein Khalili
E-mail: khalilih@tums.ac.ir

INTRODUCTION

Today, *Staphylococcus aureus* is a frequently isolated pathogen causing serious invasive infections such as soft tissue infections, endocarditis, osteomyelitis, bacteremia, septic arthritis, and nosocomial pneumonia. Available treatment options for serious invasive diseases due to *S. aureus* are limited

because of increasing antimicrobial resistance. In recent years, many isolates of *S. aureus* have evolved resistance to both synthetic and traditional antimicrobial chemotherapy.^[1] Of particular concern is the increasing frequency of methicillin-resistant *S. aureus* (MRSA). Recent surveys of *S. aureus* isolates report MRSA rates in the United States as high as 50%.^[2] The rates of MRSA are higher in patients in the intensive care unit and in those with nosocomial infections (often >60%).^[3,4] Although vancomycin is considered by many to be the mainstay for the treatment of invasive infections caused by multidrug-resistant *S. aureus*, treatment outcomes in serious infections other than skin and skin structure infections (such as nosocomial pneumonia, endocarditis, and meningitis) are less than optimal.^[5] On the other hand, there are some reports of reduced

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susceptibility of *S. aureus* strains to vancomycin.^[6-13] Therefore, the need to new treatment alternatives is obvious.

A number of methods used to detect *in vitro* synergy between antibiotics have been described; although the checkerboard and time-kill curve methods are the most widely used techniques, these methods are time-consuming. Also, the E-test method, a newer and easier technique, has availability and cost problems. In the present study, we searched for any synergistic effect between several antibiotics against drug-resistant strains of *S. aureus* with nosocomial origin using double-disk synergy test, a method routinely used for detection of extended-spectrum β -lactamase production by Enterobacteriaceae.

METHODS

This was a prospective cross-sectional study performed at Imam Khomeini Hospital of Tehran, Iran, a 1400-bed referral teaching hospital. Over a 6-month period (from October 2009 to March 2010), disk diffusion test was done for strains of *S. aureus* isolated from clinical specimens of hospitalized patients with documented nosocomial infection, according to CLSI (Clinical and Laboratory Standards Institute) guidelines.^[14] The applied antibiotic disks (Padtanteb, Iran) included oxacillin, cephalothin, clindamycin, ciprofloxacin, vancomycin, cotrimoxazole, rifampin, erythromycin, gentamicin, chloramphenicol, and meropenem. *S. aureus* ATCC 25923 was used for quality control of disk-diffusion testing. After interpretation of the results using the CLSI breakpoints,^[14] double-disk synergy test was performed for all isolates resistant to at least two of applied antibiotics to determine the effect of inactive agents against the resistant microorganism in combination with each other. Mueller-Hinton agar (Merck, Germany) was inoculated with saline suspension of fresh culture of isolated microorganism adjusted to 0.5 McFarland turbidity standard. Combinations of all possible pairs of antibiotics (to which the microorganism was resistant) were tested by placing antibiotic disks at distance of 20 mm from each other (center to center). After 16-20 hours of incubation, if synergistic effect was present among two antibiotics, an inhibition zone was formed between their disks.

RESULTS

Over the study period, of 77 patients with a type of nosocomial infection whose clinical specimens yielded

S. aureus, 41 patients had isolates resistant to at least two antibiotics (determined by disk diffusion test) that underwent double-disk synergy test. Twenty-six patients (63.4%) were male. The mean \pm SD of age for these patients was 34.6 ± 20.0 years. Table 1 shows the frequency of detected nosocomial infections. As shown, surgical site infection and pneumonia were the most frequent infections (both 29.3%) due to these resistant strains. Accordingly, from 41 clinical specimens (6 types), wound secretions and tracheal secretions were the most frequent ones (34.1% and 26.8%, respectively) followed by blood (22%), venous catheter tip (9.8%), shunt/drainage tube tip (4.9%), and sputum (2.4%).

Table 2 shows the susceptibility pattern of isolated nosocomial *S. aureus* strains determined by disk diffusion test. As shown, all isolates were sensitive to chloramphenicol. Also, with the exception of one strain, others were susceptible to vancomycin (97.6%). In contrast, susceptibility of this microorganism to other used antibiotics including cephalothin, oxacillin, clindamycin and ciprofloxacin was low.

Of all possible two-antibiotic combinations tested for isolates, only two cases of synergistic effect were detected; both effects were among rifampin and

Table 1: Frequency of detected nosocomial infections due to staphylococcus aureus

Nosocomial infection	n	%
Surgical site infection	12	29.3
Pneumonia	12	29.3
Blood stream infection	8	19.5
Wound infection	4	9.8
Venous catheter infection	4	9.8
Shunt/drain infection	1	2.4
Total	41	100

Table 2: The susceptibility pattern of isolated S. aureus strains to used antibiotics

Antibiotic	n	Susceptibility n (%)		
		Sensitive	Intermediate	Resistant
Cephalothin	41	2 (4.9)	1 (2.4)	38 (92.7)
Oxacillin	41	-	-	41 (100)
Clindamycin	41	1 (2.4)	-	40 (97.6)
Ciprofloxacin	41	1 (2.4)	-	40 (97.6)
Vancomycin	41	40 (97.6)	-	1 (2.4)
Meropenem	37	1 (2.7)	-	36 (97.3)
Chloramphenicol	27	27 (100)	-	-
Gentamicin	29	3 (10.3)	-	26 (89.7)
Rifampin	38	19 (50.0)	-	19 (50.0)
Cotrimoxazole	41	16 (39.0)	-	25 (61.0)
Erythromycin	37	2 (5.4)	-	35 (94.6)

cotrimoxazole. Both isolates had been obtained from tracheal secretions of two patients with documented ventilator-associated pneumonia (VAP) at hospital's intensive care unit (ICU). These two isolates were resistant to all used antibiotics (including rifampin and cotrimoxazole), except for vancomycin and chloramphenicol.

DISCUSSION

In our study, the results of double-disk synergy test showed synergistic effect between rifampin and cotrimoxazole against two strains of multidrug-resistant *S. aureus*. We found only one report of such an effect *in vitro* by double-disk synergy test presented by Gosbell;^[15] however, in his study, the evaluated MRSA strains had two differences with our isolates: First, they were all community-acquired strains (CA-MRSA) and second, the isolates were not multidrug-resistant strains but only resistant to penicillin and oxacillin. Therefore, our finding is of important value which shows that the combination of rifampin and cotrimoxazole could be considered for treatment of infections due to multidrug-resistant *S. aureus* after confirmation of their synergism by double-disk synergy test. In contrast to our finding, in the study of Kaka *et al.*, adding rifampin to cotrimoxazole showed a trend toward antagonism *in vitro*;^[16] also, in the study of Harvey *et al.*, the rifampin-trimethoprim-sulfamethoxazole combination was antagonistic *in vitro* against *S. aureus*.^[17] Therefore, the efficacy of such antibiotic combinations should be evaluated in clinical trials. In the study of Jemni *et al.*, all eight patients treated by the combination of cotrimoxazole and rifampin for treatment of infections due to MRSA were cured.^[18] Considering increased rate of resistance among *S. aureus* strains, it is urgent for the worldwide medical community to reexamine the clinical efficacy of older active therapies, such as cotrimoxazole, for treating severe infections due to these pathogens.

The synergism between rifampin and cotrimoxazole against resistant strains of *S. aureus* could provide a viable option for treatment of infections due to these resistant microorganisms; however, any new recommendation on the use of this combination should be based on strong evidence rather than sporadic case reports or *in vitro* studies. Also, double-disk synergy test seems to be capable of detecting the synergistic effect between antibiotics at *in vitro* level. We recommend implementation of this test as a routine antimicrobial susceptibility test in the hospitals' microbiology laboratories, at least for antibiotic-resistant microorganisms.

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AUTHORS' CONTRIBUTION

All authors contributed the idea of research, design of study, data analysis and manuscript preparation.

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