Assessment of Synergy of Antimicrobial Combinations against Bacteria Isolated from Burned Infected Patients

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Abstract

In this study, (100) wound swabs and (65) blood samples were collected from (100) patients suffering from burn wound infections with the age range between (1.5-45) years old from both sexes. The period of collection was extended from November 2011 to March 2012.

All samples were subjected to identify the bacterial cultivation, and different types of bacteria were isolated which caused burn wounds infections. The predominant bacteria was *P.aerugenusa* for gram negative, and for gram positive was *S.aureus.*

The effect of Silver sulfadiazine (SSD) and povidon iodin (PI) on bacterial isolats were studied and the results showed there is no great difference between the effect of SSD and PI on all the studied bacterial isolates.

The combination between (SSD) and (PI) and between (SSD) and some systimic antibiotics where studied and the results showed that the effects of combined antimicrobial on isolated bacteria become better than the use these antimicrobial alone.

الخلاصة

تم خلال هذا البحث جمع و دراسة ١٠٠ عينه (مسحات جلد) و ٦٥ عينة دم من مرضى الحروق (٥٩ اناث و ٤١ ذكور) و ٧٠ مسحه من ردهات الحروق وممن أحيلوا إلى مستشفى الحلة التعليمي العام (من تشرين الثاني ٢٠١١ خلال اذار٢٠١٢) . تراوحت أعمار المرضى الذين شملتهم الدراسة من (١.٥– ٤٥) سنه.

خضعت جميع العينات للزرع البكتيري للتعرف على المزارع البكتيرية واجريت الاختبارات الكيمياوية الحيوية على جميع عزلات البكتريا المسببة لالتهاب الحروق، وكانت السائدة من البكتريا السالبة لصبغة غرام هي *P.aerugenosa* والبكتريا الموجبة لصبغة غرام هي *S. aureus* .

دراست تأثيرات السلفيرسولفاديازين و البوفيدون ايوداين على بعض البكتريا المعزولة من مرضى الحروق ولوحظه عدم وجود فرق كبير بين تأثير هذين المضادين.

وتم دراسة تأثير دمج السلفيرسولفاديازين و البوفيدون ايوداين وكذلك دمج السلفيرسولفاديازين مع بعض المضادات الحيوية مثل (الاميكاسين، الازثرومايسين، السيفيبيم والاموكسلين) على العزلات البكتيريه وأظهرت الكثير من النتائج وجود تأثير اكبر على البكتريا بعد الدمج.

Introduction

Widespread application of an effective topical antimicrobial agent substantially reduces the microbial load on the open burn wound surface and reduces the risk of infection [1]. Burn units may rotate the use of various topical antimicrobial preparations on a regular basis to decrease the potential for development of antibiotic resistance [2]. The most widely used topical antimicrobial agents are Povidone iodine and Silver sulfadiazine (Silvodene, Flamazine, Thermazine, Burnazine) and Mafenide acetate (Sulfamylon) and new silver nanocrystalline dressings (Acticoat A.B. dressing, Silverlon) that are based

on the bactericidal properties of the silver ion [3].

Silver sulfadiazine is the most commonly used topical antibiotic agent for both ambulatory and hospitalized burn patients. This agent is a combination of sodium sulfadiazine and silver nitrate. The silver ion binds to the microorganism's nucleic acid, releasing the sulfadiazine, which then interferes with the metabolism of the microbe [4]. Silver sulfadiazine has excellent broad-spectrum antibacterial coverage against *Pseudomonas aeruginosa* and other gram-negative enteric bacteria, although some resistance has recently been reported [5]. This agent also has some activity against *Candida albicans*, but enhanced antifungal activity can be achieved by using nystatin in combination with silver sulfadiazine [6]. Silver sulfadiazine is only absorbed within the surface epidermal layer, which limits its effectiveness in some patients with severe injuries. Povidone iodine has broad spectrum in vitro activity but may be absorbed systemically and can be inactivated by wound exudates [7].

Materials and Methods

Collection of specimens:

Burn swabs(100) and blood samples(65) were collected from (100) patients suffering from burn infection from both sexes who are admitted to burn ward in AL_Hilla general hospital and environmental swabs from burn ward were also obtained. The period from November 2011 to March 2012.

The bacterial isolates were identified after staining the bacteria with gram stains, traditional biochemical tests were done to reach the final identification according to [8,,9].

The Effect of Silver Sulphadiazine and povidone-iodine, Incidin alone or in combination between them on Bacterial Growth:

This is done according to Starodub and Trevors[13].

Effect of combination between SSD and some systemic antibiotic on bacterial isolates:

This is done according to (Al-saeed [14] personal communication)

a. 1g of (1% SSD) was dissolving in 100 ml of Mueller-Hinton agar media after autoclaved at 121C^{o} for 15 min, and then cold to 45 ^{o}C.

b. The inoculums that used in this test were prepared by adding (5) isolated colonies grown on blood agar plate to 5 ml of nutrient broth and incubated at 37^{o}C for 2 hours and compared with (0.5) McFarland standard tube (and this done according to the bacterial specieses).

c. A sterile swab was used to obtain an inoculums from the bacterial suspension, this inoculums was streaked on MHA plate with (SSD) and left to dry.

d. The antibiotic discs were placed on the surface of the medium at evenly spaced intervals with flamed forceps and incubated for (24-48) hours at 37^{o}C.

The antibiotics disc are:

AK= Amikacin , **AZM**= Azithro-mycin **, FEP**= Cefepime , **AX**= Amoxiccillin

Results and Discussion

Effects of silver sulphadiazine (SSD), Povidone iodin (PI), Incidin alone or in combination on bacterial isolates:

Defferent bacterial species were isolated from burn infected patients as shown in table (1) and table (2) that demonstrated the frequency of bacteria in skin swab, blood and burn ward cultures, it is clear from the total number of isolates that gram negative bacteria are more frequent than gram positive type. This agrees with Kamel and El-Megeed [12], who found that gram negative bacteria represent about 65% of micro-organisms that cause burn wound infection and that this type

of bacteria has assumed a primary lethal role among the cases of burn wound infection and septicaemia.

Table 1 Frequency of Gram negative Bacteria

Bacterial Isolates	Skin	Blood	Burn unit
	Frequency	Frequency	Frequency
Pseudomonas aeruginosa	29	13	17
Enterobacter spp.	13	7	5
Escherichia coli	14	3	5
Acinetobacter baumannii	11	2	4
Klebsiella pneumoniae	11	1	4
Proteus spp.	4	——	2
Total	٨٢	٢٦	37

Table 2 Frequency of Gram Positive Bacteria

Bacterial Isolates	Skin	Blood	Burn unit
	Frequency	Frequency	Frequency
Staphellococcus aureus	16	7	17
Staphellococcus epidermidis	4	2	——
Enterococcus faecalis	5	2	4
Streptococcus spp.	3	2	——
Total	28	13	21

The effects of silver sulphadiazine (SSD 1%) and povidone-iodine (PI) on the most frequent bacteria isolated from burned patients were tested in this study. These effects are shown in figure (1) and in table (3). There is no great difference between the effect of SSD and povidone on all the studied bacterial isolates. The most affected bacterial species by SSD is the *E. faecalis* and the least susceptible is *S. aureus* and *Enterobacter spp.* , whereas the most affected bacterial species by povidone-iodine is *S. epidermidis*, *E. faecalis* and *A.baumannii* and the least affected are *Enterobacter spp*.

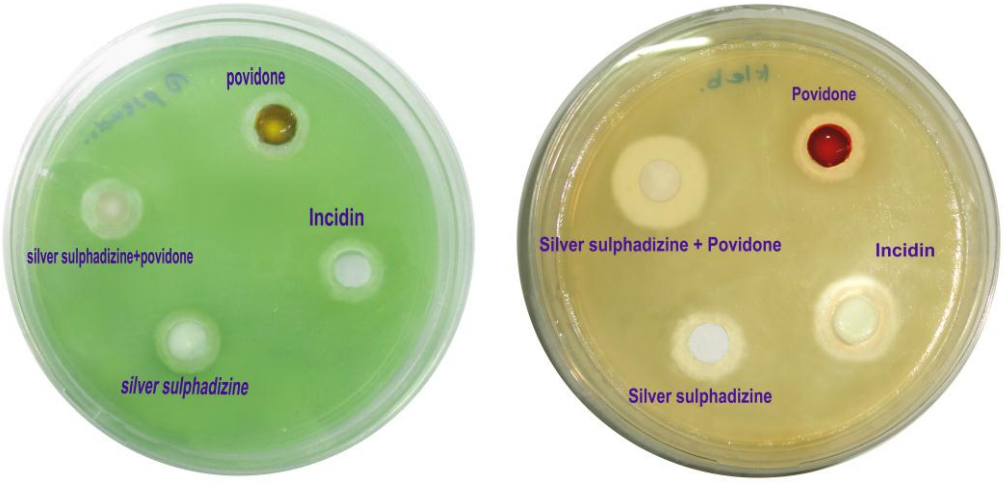

Figure 1 zone of inhibition caused by SSD, PI, Incidin and combination between them on bacterial isolates(*pseudomonas* on left and *Klebsiella* on right).

Noronha and Almeida [13] pointed out that SSD was the drug of choice for prophylaxis of burn wound infection in most burn patients. The clinical trials have shown that SSD is efficacious in reducing bacterial numbers and delaying burn wound colonization with gram negative bacteria [14]. The mechanism of the antimicrobial action of SSD (Ag-s) has not been fully elucidated. Nonetheless, it is thought to act via inhibition of DNA replication and modifications of the cell membrane and cell wall. In addition, silver ions in Ag-S interfere relatively non-specifically with a number of enzymes, including some involved in the synthesis of the bacterial cell wall [15].

The biological substances secreted by the damaged skin and the damaged or dead tissues itself may interfere with the action of SSD or PI on bacterial isolates colonizing the skin and may reduce their effectiveness. Drost *et al* [16] pointed out that bacteria were protected by dead tissue in to which antibiotics diffuse poorly and they mentioned that local antibiotics are of little value in the treatment of infected burns as long as dead tissue is still present, and so they mentioned that the mainstay of treatment is the rapid removal of dead tissue by surgical excision in the form of immediate burn wound excision with immediate skin graft. Al-Akayleh [17] found that 0.5% silver nitrate and 1% SSD had an effect in the reduction of the incidence of burn wound sepsis.

povidone-iodine (PI) is a widely used topical antimicrobial agent in cases of burn-injured patients. PI contains 10% povidone-iodine in a water-soluble base with a pH value of 1.5 to 3.5, and yields 1% available iodine. The free iodine is released at a slow rate from its complex at the treated site, where it acts as a bactericidal and fungicidal agent, especially in longer protracted cases [18].

Steen [19], described PI to be superior to silver nitrate in reducing the number of bacteria in burn wounds.

The combination between povidone-iodine (PI) and silver sulphadiazine (SSD) were also studied, and the results shown in table (3). Topical antimicrobial combination was superior to the use of SSD or PI alone. There are clear effect on some bacteria isolate (*Streptococcus spp.*, *E. faecalis*, *E.coli*, *K. pneumoniae*, and *Enterobacter spp.*) after combination and these effects suggested as a result of increase in the inhibition zone as shown in figure (1). The increase in the

inhibition zone may results from the synergistic effect between these two ointment antimicrobial. Chernish and Aaron [20] demonstrated that antibiotic combinations that exhibit synergistic effects represent a valuable addition to standard treatment regimens and may overcome treatment failure.

The assessment of the in vitro synergy of antimicrobial combinations will provide more options for successful antibiotic therapy in the clinical setting [21].

Regarding, the effect of incidin against bacterial isolates was also investigated as shown in table (3). The most bacteria effected by this disinfectant was *S. aureus* while the lowest one were *P. aeruginosa* and *E. coli*. This results nearly compatible whit Carraher and Louda [22], who found that incidin (Tri-butyltinoxide) is extremely effective in controlling bacteria in hospitals, such as *S. aureus.*

(Incidin) is a mixture of tri-*n*-butyltin benzoate and formaldehyde. Danish *et al* [23] demonstrated that incidin is highly effective disinfectant against gram-negative and gram-positive bacteria which may be used on open areas posing a risk of infections such as hospital floors.

Tabel-3 The mean of inhibition zone (mm) caused by effects of SSD, PI, Incidin and combination between them on bacterial isolates

Bacterial isolates (no.)	SSD 1%	PI	SSD +PI	Incidin
S. aureus (3)	12	14	14	24
S. epidermidis (3)	13	15	15	22
Streptococcus spp. (3)	14	14	18	20
E. faecalis (3)	18	15	27	16
P. aeruginosa (3)	13	14	13	13
A. baumannii (3)	14	15	15	18
E. coli (3)	14	14	21	14
K. pneumoniae (3)	14	14	25	16
Enterobacter spp. (3)	12	13	22	22
Proteus spp. (3)	14	14	14	22

Effect of combination between SSD and some systemic antibiotic on bacterial isolates:

Some systemic antibiotics were tested on bacterial isolates alone and in combination with silver sulphadiazine (SSD) as shown in table (4).

The combination of a topical plus a systemic drug was compared with a systemic antimicrobial only and the results showed that the combination is superior to use the SSD or systemic antibiotic alone and these result demonstrated in table (4), and figure

(2). So, using of combination of a topical plus a systemic drug is very important for burned patients because it may reduce the septicemia. Liberati *et al* [24] indicate that an antibiotic prophylaxis regimen including a combination of topical and systemic antibiotic significantly reduces overall mortality.

The combination of topical and systemic antibiotics is now the standard against which new treatments should be tested. A logical next step for future trials would seem to be the comparison of this protocol against a regimen based on a systemic antimicrobial only [24].

Table 4 The mean of inhibition zone (mm) caused by effect of SSD plus systemic antibiotic on bacterial isolates.

Bacterial isolates (no.)*	Systemic antibiotic alone				SSD alone	SSD (0.01%) +			
	AK	AZM	FEP	AX		AK	AZM	FEP	AX
S. aureus (3)	22	11	8	0	12	S	30	14	0
S. epidermidis (3)	19	9	0	0	13	S	25	0	0
Streptococcus spp.(3)	24	12	7	11	14	S	45	9	34
E. faecalis (3)	24	9	0	8	18	S	15	7	30
P. aeruginosa (3)	20	12	0	9	13	S	25	0	17
A. baumannii (3)	26	7	12	0	14	S	23	18	18
E. coli (3)	25	7	0	11	14	S	20	8	14
K. pneumoniae (3)	11	19	7	0	14	21	24	8	16
Enterobacter spp. (3)	23	18	9	7	12	S	29	11	14
Proteus spp. (3)	28	9	14	9	14	S	40	32	22

*non of these bacterial isolates were grown after 24hrs. , **S**= sensitive

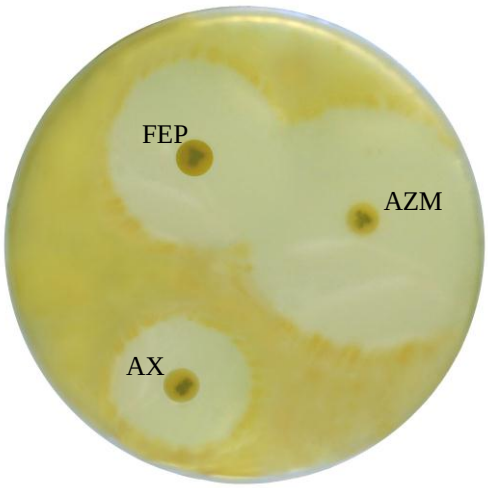

Figure 2 Effects the combination of SSD and systemic antibiotic on bacterial isolates (*proteus ssp.*).

Conclusions

1- Gram negative bacteria are more predominant as causative agents for burn infections than the gram positive type.

2- The most affected bacterial species by SSD is the *E. faecalis* and the least susceptible is *S. aureus* and *Enterobacter spp.*

3- The most affected bacterial species by povidone-iodine is *S. epidermidis*, *E. faecalis* and *A.baumannii* and the least affected are *Enterobacter spp*.

4- Topical antimicrobial combination was superior to the use of SSD or PI alone.

5- The combination of a topical plus a systemic drug is superior to use the SSD or systemic antibiotic alone.

Recommendation

Molecular research is needed to clarify the effect of SSD and PI on bacteria isolates from burnd patients.

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