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STUDY THE INHIBITORY EFFECTS OF *BACILLUS SUBTILIS* FILTRATES ISOLATED FROM IRAQI SOIL IN COMBINATION WITH MIC OF CIPROFLOXACIN AND IMIPIMEN ON *ACINETO-BACTER BAUMANNII* ISOLATED FROM WOUNDS AND BURNS

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ABSTRACT

The study were performed to detection the inhibitory effect of filtrates *Bacillus subtilis* isolated from soil against *Acinetobacter baumannii* Isolated from wounds and Burns .4 isolates of *B. subtilis* showed inhibitory activity of filtrate without concentrated and with concentrated against 5isolates of *A. baumannii*. Antibiotic susceptibility test of *A. baumannii* was performed and MIC determined to the most effective antibiotic ciprofloxacin and imipimen,and studying the inhibitory effect of the combination the filtrates *B. subtilis* and sub MIC of these antibiotics against *A. baumannii*. The result showed that the filtrate of isolate *B.subtilis* against *A .baummani* has inhibition effect, when using bacterial filtrate alone, and these activity was increased when using sub MIC of ciprofloxacin and imipimen in combination with filtrates of *B. subtilis*. Separately. The beast antimicrobial activity was obtained by using Imipenem against *A. baummani* in combination with filterates.

KEYWORDS: *Bacillus subtilis, Acinetobacter baumannii, A. baumannii and B. subtilis.*

INTRODUCTION

antibiotics production by some bacteria plays a major role in disease suppression. Mechanism of resistance in clinical infection reflects very serious problem in the treatment of pathogenic microbes. Serious bacterial and fungal infections are increasingly recognized as important causes of morbidity and mortality.[1]

To confront the growing antimicrobial resistances, modern medicine focus natural products for novel antibiotics and antimicrobials. The model system for gram-positive organisms, *Bacillus* species are able to produce more than two dozen antibiotics with different structures and functions depending on the ecological niche or induced systematic resistance Bacillus isolates are rather well known for the production of a vast array of structurally unrelated antimicrobial compounds, which include lipopeptides like iturin, surfactin, fengycins, bacteriocins, and bacteriocin like inhibitory substances.[2]

Acinetobacter species cause pneumonia, skin and wound infections, bacteremia, and urinary tract infections while they have shown resistance to various antibiotics. Infections due to resistant *Acinetobacter baumannii* isolates cause difficulties in treatment Management of multidrug-resistant *Acinetobacter* spp. infections is a great challenge for physicians and clinical microbiologists. Its ability to survive in a hospital milieu and its ability to persist for extended periods of time on surfaces makes it a frequent cause for healthcare-associated infections and it has led to multiple outbreaks.[3] It causes a wide spectrum of infections . Among all the antibiotics only few are very effective because most of the antibiotics show side effects. And some antibiotics show adverse reactions when they are used in high doses. To reduce these side effects combination of drugs were discovered. The combination effect shows the more activity than the single drug. When two drugs combined they give either additive effect or synergistic effect.[4]

The aim of this study was to isolate, *Bacillus subtilis.* from soil and investigate antimicrobial activity against *Acinetobacter baummani* isolated from burn and wounds by using filtrate of *B. subtilis* alone and in combination with sub MIC of some antibiotics .

MATERIALS AND METHODS

Bacterial isolate: soil samples collected from different areas of Iraq (Baghdad, Najaf, Karbala ,Babel) *B. subtilis* were isolated. *A bummani* were isolated from samples collected from burn and wounds infections. Identification of isolated bacteria species, were done according to the morphological and biochemical test.[5]

Determination of antimicrobial activity of antibiotics against *A. baummani*

Antibiotic susceptibility test was Performed to the isolates of *A. baummani* using Kirby Bauer method on mueller –Hinton agar plates- antibiotic discs used are as follows), Penicillin-G), Chloramphenicol, Imipenem, oxacillin, tobromycin, Nitrofurendtin,

Ceftazidime, co-trimoxazole, clindamycin, nalidixic acid, tetracycline, amikacin, ticarcillin, cefoxitin, Ciprofloxacin. MIC was determined by using agar dilution method to most effective antibiotic against of *A. baummani* ciprofloxacin and imipimen.as described in.[5]

Inhibitory effect of *B.subtilis* filterate against *A .baummani*

The determination of the inhibitory effect was carried out according to the agar-well diffusion method. To prepared filterate of *B.subtilis*, bacteria were cultured on NB medium and incubated at 37◦ c for 4 days in agitating incubator. The *Bacillus* cultures were centrifuged at 6000*g* for 15 min to remove cell debris. After centrifugation, supernatant pass through Millipore filter paper(0.22 µl) to obtained the bacterial filtrate . Suspensions (100 µl) of *A. baummani* cultured at 37c◦ for 24 h were spread on the plates, and wells of 6 mm diameter were cut using a cork borer..Filtrate of *B.subtilis* (100 µl) was then filled into the wells of agar plates inoculated with *A. baummani*. 6mm diameter well, 100µl of D.W. was placed as a negative control. The inoculated plates were incubated for 24 h at 37◦c, and the diameter of the inhibition zone was measured with calipers as mm.[6] To increase their activity, *B. subtilis* filtrates were concentrated by using lyophilizere system, then resuspension by using 10 and 5 ml of distilled water for one and two-fold concentration. Adding (100µl) of one and two-fold concentration separately in the well as mentioned above instead the unconcentrated filtrates.[7]

Inhibitory effect of *B.subtilis* filtrates in combination with sub MIC of ciprofloxacin against *A .baummani*

The antibacterial activity of *B.subtilis* filtrate in combination with sub MIC of ciprofloxacin was carried out using agar well diffusion method to detect influence the combination of filtrate and sub MIC of ciprofloxacin against *A. baummani*. Three different concentration of *B.subtilis* filtrate used (unconcentrated and concentrated one and two-fold concentration) in separated by mixing (50 µl) sub MIC of ciprofloxacin with (50 µl) of *B.subtilis* filtrate in the agar-well diffusion method.[7]

Inhibitory effect of *B.subtilis* filtrates in combination with sub MIC of Imipenem against *A .baummani*

The experiment done as mentioned above by adding (50 µl) sub MIC of,,Imipenem instead (50 µl) sub MIC of ciprofloxacin.

RESULTS AND DISCUSSION

As a result of the identification tests, 4 isolates of *Bacillus subtilis* Isolated from soil and investigate their antimicrobial activity against 5 isolates of *Acinetobacter baummani* isolated from burn and wounds infections.

The result of susceptibility test of *A. baumannii* to commonly used antibiotics, indicated that bacteria showed highly resistance to most antibiotics (fig.1). highly levels of resistance(100%) were recorded to Penicillin -G, oxacillin, tobromycin, cefoxitin, cefoxitin, ticarcillin and (80%) to Chloramphenicol, nalidixic acid, Nitrofurendtin, Ceftazidime, (60%) were recorded to co-trimoxazole co-trimoxazole, and the resistance of *A. baumannii* isolates (40%) were recorded to Imipenem, Ciprofloxacin.

MIC was determined to ciprofloxacin and imipimen most effective antibiotic against *A. baummani*. MIC was defined the lowest antimicrobial drug concentration at which there was no growth was considered as the minimum inhibitory concentration (MIC). The results were interpreted according to CLSI Clinical and Laboratory Standards Institute criteria.[8] The bacteria considered to be sensitive when the MIC value less .then, cut –off value.[9]

In our study the *A. baumannii* isolates showed highly sensitive to Ciprofloxacin that 4 out of 5 isolates can grow in concentration less than cut –off value (≥ 15 µg/mL). Imipenem showed less effective than Ciprofloxacin, 2 out of 5 isolates can grow in concentration less than cut –off value(≥ 16 µg/mL). Table 1

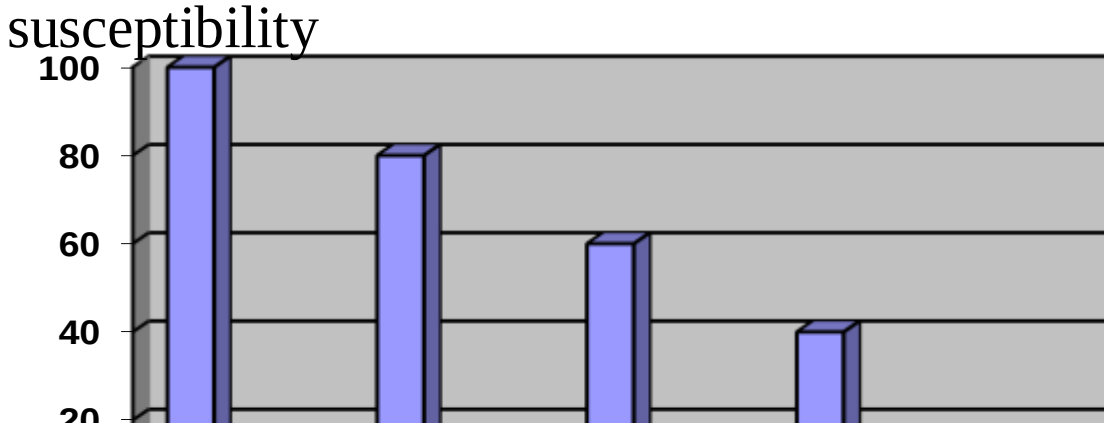

Fig.1: percentage of Antibiotic susceptibility test *of A. baumannii* isolates

A: Penicillin-G, oxacillin tobromycin , cefoxitin , cefoxitin , ,ticarcillin

B: Chloramphenicol, nalidixic acid , Nitrofurendtin ,Ceftazidime

C: co-trimoxazole co-trimoxazole D: Imipenem, Ciprofloxacin

These results agreement with many studies reveld that Many of the commonly used antibiotics, including cephalosporins, ureidopenicillins,quinolones, gentamicin and cotrimoxazole,were virtually useless against this pathogen[10]

The mechanisms of antimicrobial resistance in *A. baumannii* generally falls into three broad categories: 1- antimicrobial-inactivating enzymes, 2- reduced access to bacterial targets (due to decreased outer membrane permeability caused by the loss or reduced expression of porins, over expression of multidrug efflux pumps) and 3- mutations that change targets or cellular functions (alterations in penicillin-binding proteins; PBPs). A combination of several mechanisms may be present in the same microorganism, as has also been observed in other gram-negative bacteria.[3]

Table -1: MIC value of ciprofloxacin and imipimen for *A. baumannii* isolates (µg/mL)

***A.baumannii* isolates**	**MIC value Ciprofloxacin**	**MIC value Imipenem**
Ab 1	>1024	32
Ab 2	1	2
Ab 3	4	>1024
Ab 4	4	1
Ab 5	4	>1024

Some isolates of *B.subtilis* show antimicrobial activity against *isolates* of *A. baummani*, but other isolates did not show antimicrobial activity (Table 2). The isolate (Bs1) shows inhibitory effect against *A. baummani*, while the isolate (Bs3) did not show any antimicrobial activity when used the filtrate of *B. subtilis* without concentration. The diameter of inhibition zone of Bs1 was (0 -10) mm and to Bs3 was (0)mm. these activity was increased when concentrated the filtrate one and two fold. the diameter of inhibition zone of Bs1 filtrate one and two fold concentration was (10-16)mm and (19-22)mm respectively, and the diameter of inhibition zone of Bs3 was (7-10)and (10-15)mm respectively . Table- 2.

The results agreement with many other research which recorded The secondary metabolites produced by several species and strains of the genus *Bacillus* have been found to show antimicrobial activity against different pathogens,and the .production of this compounds at late exponential growth phase.[11], [12]

The antimicrobial activity was observed against the gram-positive bacteria like *Staphylococcus aureus, Streptococcus pyogenes,*and *Enterococcus faecalis*and pathogenic

yeasts like*C. albicans, C. glabrata, C. krusei, and Cryptococcus neoformans*(2). In one study, Hei *et al. (2006)* mentioned that the antimicrobial activity of The ultrafiltered concentrate was tested against 32 strains belong to the gram-positive and negative bacteria and found that concentrated filtered be effective against wide spectrum of bacteria examined in the investigation.(6)

Table 2: diameter of inhibition zone in millimeter (mm) of *B.subtilis* isolates against *A. baummani* isolates.

	isolate	Acb 1	Acb 2	Acb 3	Acb 4	Acb 5
Bs1	unconcentrated filtrate	0	9	7	10	7
	filtrate one fold concentration	10	16	15	15	13
	filtrate two fold concentration	20	20	20	22	19
Bs2	unconcentrated filtrate	0	8	0	7	0
	filtrate one fold concentration	9	15	10	13	10
	filtrate two fold concentration	15	20	18	19	17
Bs3	unconcentrated filtrate	0	0	0	0	0
	filtrate one fold concentration	7	10	7	9	9
	filtrate two fold concentration	10	13	10	12	15
Bs4	unconcentrated filtrate	0	0	0	7	0
	filtrate one fold concentration	7	10	9	13	9.5
	filtrate two fold concentration	13	15	14.5	19	17

Detect the influence of *B.subtilis* filtrate in combination with sub MIC of ciprofloxacin and Imipenem

Antimicrobial activity of *B.subtilis* filtrates in combination with sub MIC of ciprofloxacin and Imipenem against 5 isolates of *A baummanii* isolated from burn and wounds infections was determined using agar well diffusion method .the results showed good antimicrobial activity against *A baummani*i when using filtrates in combination with sub MIC of ciprofloxacin (Table- 3) the higher diameter of inhibition zone was of Bs1 isolate (0-27) mm and for Bs3 was (0-20) mm .these activity was increased when used concentrated filtrate one and two fold separately in combination with sub MIC of antibiotics and The result also showed that the Imipenem were more effective on the *A .baummani* isolates under test in comparison to the ciprofloxacin . The higher antimicrobial activity was

obtained by using Imipenem, and the higher diameter of inhibition zone was of Bs1 isolate (7-30) mm and for Bs3 was (0-15) mm(Table- 4)

Table -3: diameter of inhibition zone in millimeter (mm) of *B.subtilis* filtrate in combination with sub MIC to ciprofloxacin against *A .baummani* isolates.

	isolate	Acb 1	Acb 2	Acb 3	Acb 4	Acb 5
Bs1	unconcentrated filtrate	0	15	10	15	10
	filtrate one fold concentration	10	20	19	20	15
	filtrate twofold concentration	20	25	25	27	22
Bs2	unconcentrated filtrate	0	10	7	10	7
	filtrate one fold concentration	9	20	15	15	15
	filtrate twofold concentration	15	25	22	23	20
Bs3	unconcentrated filtrate	0	7	0	9	0
	filtrate onefold concentration	7	13	10	15	13
	filtrate twofold concentration	10	17	15	20	19
Bs4	unconcentrated filtrate	0	9	9	15	7
	filtrate onefold concentration	7	15	15	17	13
	filtrate twofold concentration	13	20	17	23	19

Table 4: diameter of inhibition zone in millimeter (mm) of *B.subtilis* filtrate in combination with sub MIC to Imipenem against *A .baummani* isolates.

	isolate	Acb 1	Acb 2	Acb 3	Acb 4	Acb 5
Bs1	unconcentrated filtrate	13	15	7	15	7
	filtrate one fold concentration	20	20	15	25	13
	filtrate two fold concentration	25	30	22	30	19
Bs2	unconcentrated filtrate	10	15	0	13	0
	filtrate one fold concentration	15	20	10	20	10
	filtrate two fold concentration	20	27	18	30	17

Bs3	unconcentrated filtrate	7	10	0	10	0
	filtrate one fold concentration	10	15	7	15	9
	filtrate two fold concentration	19	18	10	20	15
Bs4	unconcentrated filtrate	10	10	0	15	0
	filtrate one fold concentration	13	20	9	20	9.5
	filtrate two fold concentration	20	22	14.5	25	17

Our result showed thatThe filtrate of the isolate *B.subtilis* against *A .baummani* has inhibition effect, when using bacterial filtrate alone and these activity was increased when using sub MIC of antibiotics . The the beast antimicrobial activity was obtained by using Imipenem against *A .baummani* isolates instead ciprofloxacin . New antibacterial agents are required for the treatment of infections caused by multidrug-resistant (MDR) Acinetobacter spp.[13] other study confirmed that Imipenem, ofloxacin, and ciprofloxacin had the best antimicrobial activity with minimum inhibitory concentrations, and the Combinations of two antimicrobial agents. ceftazidime, aztreonam, imipenem, or ciprofloxacin with amikacin showed either synergistic effects or partial synergistic effects.[14]

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