INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

BACTERIOLOGICAL PROFILE OF BURN PATIENTS AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BIO FILM FORMING ISOLATES TOWARD ANTIBIOTIC AND PLANT EXTRACTS

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Article Received on: 06/02/19 Approved for publication: 01/04/19

DOI: 10.7897/2230-8407.1005157

ABSTRACT

Objective: Bio film is an association of micro-organisms in which microbial cells adhere to each other on a living or non-living surfaces within self-produced matrix. Bacteria are the most common microorganism found in a bio film and they are very infectious. Antibiotics are a normal choice to treat bacteria, but because of the bacterial resistance for the usual antibiotics, we aimed to examine some natural plants extracts effect on bio film. Methods: A total of 56 specimens were obtained from patients suffering from burn wound infections. Eight types of bacteria were isolated and identified by standard biochemical tests used for clinical diagnosis. The susceptibilities of all strains against Penicillin G, Trimethoprim, Gentamicin, Vancomycin, Tetracycline and Ciprofloxacin were assessed by Disc-Diffusion method. Assessment of antibacterial activity of some plant extract on bio film forming bacteria was done by agar well diffusion method. Results: 57.14 % of swabs were strong bio film forming bacteria, 25 % were moderate, 10.72 % were weak and 7.14 % were no bio film forming. The single bacterial isolates were 42.86 %, mixed isolates were 57.14 %. The most common isolated pathogen was *Pseudomonas aeruginosa* (23.41 %) as well as *Escherichia coli* (19.14 %). The isolates were highly resistant to Penicillin G, Vancomycin, Trimethoprim and Tetracycline (91.0 %, 87 %, 85.7 % and 82.1 %) respectively and sensitive to Ciprofloxacin. The results show that fresh juice of *Allium sativum* has strong antibacterial activity against the isolate, hot water extract of *Myrtus communis* has weak antibacterial activity while fresh juice of *Allium cepa* has no antibacterial activity against the isolates.

Keywords: Bio film, burn patients, *Allium sativum*, *Myrtus communis*, *Allium cepa.*

INTRODUCTION

Bio films can be described as collection of cells attached to a surface and established on a self-produced matrix of extracellular polymeric substances (EPS). They are able of modifying growth rate, furthermore bio film organisms reproduce genes that plank tonic organisms of the same species do not[1,2]. Bio film consists of 2-5 % microbial cells and extracellular polymeric substances correspond to 1-2 % of matrix composition, while the rest is water, so is highly absorptive and porous structure[3]. Bacteria is the most common microorganism found in a bio film, the main characteristics which give bacteria large capacity for bio film production are: Production of extracellular substances and structures, the high reproduction rates and great adaptability with environment conditions[4]. *Pseudomonas*, *Bacillus*, *Staphylococcus*, *Flavobacterium* and *Alcaligenes* are the most common bio film producer genera[5]. However, bio film formation has been best studied in members of the *Pseudomonas* genus because they are amongst the most diversified bacterial species in the environment, they are involved in medical conditions like cystic fibrosis and they are known to be good bio film producers[6]. Comparative to the bio film studies and characterization using *Pseudomonas* species, garlic has proved its ability to act as a potent antibiotic against various Gram's positive, Gram's negative and acid fast bacteria[7,8]. Some studies showed that garlic antibacterial action depends on allicin, garlic oil and diallyl sulfides[9,10], They interference of these compounds with bacterial cell wall synthesis by increase bacterial membrane permeability or by inhibition of bacterial protein synthesis at 30S subunit of ribosomes[11,12].

MATERIALS AND METHODS

This study adhered to tenets of the Declaration of Helsinki and was approved by the Ethics Committee of Department of Pharmacy, Al Safwa University, Kerbala, Iraq. A written consent was taken from all the study subjects, this study was in accordance with ICH GCP Guidelines A total of 56 swabs were taken from patients suffering from burn wounds infection 34 swabs were taken from males and 22 females, who were admitted to the burn unit of Al- Hussain Medical City in Karbala, during a period extending from 1-28 February 2017.

Isolation and Identification of microorganisms

Twenty eight samples were collected from burns wound patients; isolation and identification were done by standard biochemical tests used for clinical diagnosis. The phenotypic tests used were described in The Bergey´s Manual of Determinative Bacteriology.

Antibacterial susceptibility testing

The susceptibilities of all strains to Penicillin G, Trimethoprim, Gentamicin, Vancomycin, Tetracycline and Ciprofloxacin were assessed by Disc-Diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) and the strains were classified as Resistant (R), Intermediate (I) or Sensitive (S), according to the zone table, constituted by Clinical and Laboratory Standards Institute[13].

Bio film formation capability in bacterial isolates

The determination of bio film formation in all strains was performed by Crystal Violet Binding Assay described at[14]. Strains were classified as follows[15].

A. OD = ODc = non bio film forming bacteria
B. ODc < OD ≤ 2ODc = Weak bio film forming bacteria
C. 3ODc < OD ≤ 4ODc = Moderate bio film forming bacteria
D. 4OD < ODc = Strong bio film forming bacteria

Plant collection and Processing

500 gm of each of *Allium sativum* bulb*, Allium cepa* bulb and *Myrtus communis* leaves were bought from local market at AL-hur city. Plants were cleaned and dried and grinded into powder by sterile dry blender. Hot water extracts done in[16].

Assessment of antibacterial activity of plant extract on bio film forming bacteria (Agar well diffusion method)

A volume of 10 ml of Mueller-Hinton agar media was poured separately into each sterile petri dish. Test tubes containing sterile normal saline were inoculated with colonies of activated isolates. The turbidity of suspension was adjusted to read absorbance of 0.080 at 620 nm; this absorbance represents 10^8 bacteria/ml on McFarl and turbidity standard. 100 µl of this suspension was spread on the agar using sterile spreader and the plates were left aside for 10-20 minutes at room temperature. Several wells of 6 mm diameter were created on the agar at convenient distances (about 2.5 cm between each other). In addition a similar volume of distilled water (control) was added into one well. Then the plates left for 15 minutes at room temperature and then incubated aerobically for 24 h at 37°C. The diameter of inhibition zone was measured across each well. Resistance of bacteria to the tested agent was indicated when there was no zone of inhibition[17].

The statistic method used for analyzing the result of this study was Chi-square test. *P* value ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

All the 56 patients involved in this study stayed for 48 hours at least at burn unit of Al- Hussain Medical City. Sixteen patients were third degree burns and the others were second degree with age range between 11 months and 83 years.

Our results showed that the most common isolated pathogen of burn patients is *Pseudomonas aeruginosa* with ratio of 23.41 % which agrees with Alwan who found that *P. aeruginosa* was the most common bacteria in Al- Kendi teaching hospital[18]. Ramakrishnan also found that *P. aeruginosa* is the predominant bio film formation bacteria in burn wound infection in a pediatric hospital in Chennai, India[19].

Table1: Bacterial isolates from burn patients

Test bacteria	Frequency	Percent %
Pseudomonas aeruginosa	22	23.41
Escherichia coli	18	19.14
Proteus vulgaris	16	17.02
Citrobacter freundii	14	14.89
Corynebacterium xerosis	8	8.52
Staphylococcus aureus	8	8.52
Streptococcus pyogenes	6	6.38
Bacillus megaterium	2	2.12
Total	94	100

This study found that *Escherichia coli* comes in the second place regarding its percent in the isolates (19.14 %) and that is agree with Langaee and co-workers[20]. Our results showed that *P. aeruginosa* and *E. coli* are the most frequent bacteria in burn wounds. Gram negative bacteria like *Proteus vulgaris*, *Citrobacter freundii* and *Corynebacterium xerosis* were more frequent than Gram positive bacteria like *Staphylococcus aureus* and *Streptococcus pyogene*s and this opposite to Mohammed results who found that *Staphylococcus aureus* was more frequent than *Proteus* spp. in Al-Yarmouk teaching hospital, Baghdad[21]. Mohammed and co-workers also found that *Staphylococcus aureus* is the most predominant isolate from wound infections among inpatients and outpatients attending the University of Gondar Referral Hospital, Northwest Ethiopia[22].

In this study the single bacterial isolates were 24 of 56 (42.86 %) and the mixed isolates were 32 of 28 (57.14 %). Alwan and co-workers also found that 40 % of bacterial isolates from burn wound were mixed while Asati and Chaudhary found that 61.6 % of bacterial isolates was single bacterial infection samples[18,23]. All the isolates were placed in four different categories (as shown in Table 2): strong, moderate, weak and no bio film producers. 57.14 % of swabs were strong bio film forming bacteria, 25 % were moderate, 10.72 % were weak and 7.14 % were no bio film forming. The mix isolate was the most strong bio film swabs. Communication among bacteria is essential for initial colonization and successive bio film formation on the enamel surfaces[24].

Table 2: Bio film categories and frequency in the current study

categories	Frequency	Percent %
Strong	32	57.14
moderate	14	25.0
weak	6	10.72
no bio film	4	7.14
Total	56	100

The antibiotic susceptibility of these isolates revealed that 91.0 % of isolates were resistant to penicillin G; it also showed high resistance to Vancomycin 49 (87.5 %), Trimethoprim 48 (85.7 %) and Tetracycline 46 (82.1 %), moderate resistance was shown to Gentamicin 63 (64.2 %). Ciprofloxacin was the most influential antibiotic on bacterial species with 1.8 % resistance percentage. As shown in Table 3 and according to CLSI protocol 2014[21]. It was also found that Ciprofloxacin resistance is 41.7 %, 53.8 % against *Pseudomonas* spp. and *Klebsiella* spp. that were isolated from burn wound. Recent study found that strong bio film forming gram-positive bacteria isolates showed 93.5 % resistance to penicillin while no resistance was observed against vancomycin[23]. In the same study *P. aeruginosa* isolates show 49.2 % resistance to ciprofloxacin. Another study found that *P. aeruginosa* has 27.4 % resistance to ciprofloxacin[25]. Table 3 shows the antibiotics susceptibility testing in the study.

Table 3: Antibiotics susceptibility testing in the current study

Antibiotic	Symbol	Dose (mg)	Frequency of bacterial resistance	Percent %
Penicillin G	P	10	51	91.0
Vancomycin	VA	10	49	87.5
Trimethoprim	TMP	10	48	85.7
Tetracycline	TE	10	46	82.1
Gentamicin	CN	10	36	64.2
Ciprofloxacin	CIP	10	1	1.8

Table 4: Antibacterial activity of plant extraction testing in the current study

Test bacteria	Inhibition zone (mm)		
	Allium sativum	***Allium cepa***	***Myrtus communis***
Pseudomonas aeruginosa	25	R	10
Escherichia coli	20	R	R
Proteus vulgaris	25	R	R
Citrobacter freundii	30	R	R
Corynebacterium xerosis	28	R	R
Staphylococcus aureus	20	R	9
Streptococcus pyogenes	18	R	9
Bacillus megaterium	26	R	R

R: Resistant

On the other hand, and on the same isolates, onion (*Allium cepa*) showed no antibacterial activity against the isolate, while *Myrtus communis* has low values compared with *Allium sativum. Myrtus communis* has 10 mm inhibition zone with *Pseudomonas aeruginosa* and 9 mm with both *Staphylococcus aureus* and *Streptococcus pyogene*s. Azu and co-workers in their study found that raw extract of onion has no antibacterial activity against *S. aureus* and weak antibacterial activity against *P. aeruginosa* while Besufekad and co-workers found that water extract of *Myrtus communis* has no antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus*[26,27].

Table 5: Garlic and Ciprofloxacin susceptibility testing in the current study

Bacteria	Inhibition zone (mm)	
	garlic	**Ciprofloxacin**
Pseudomonas aeruginosa	21	20
Escherichia coli	23	19
Proteus vulgaris	24	17
Citrobacter freundii	23	11
Corynebacterium xerosis	25	12
Staphylococcus aureus	25	15
Streptococcus pyogenes	18	13
Bacillus megaterium	26	19
Average	23	16

Sensitivities of all isolate to Garlic as determined by the diameter of the zone of inhibition in millimeter was tested and compared to Ciprofloxacin (the most activate antibiotic in the study) which is a broad spectrum antibiotic of fluoroquinolone that act by inhibition of DNA gyrase, these bacteria were more sensitive to garlic compared to Ciprofloxacin in the average of inhibition zone caused by garlic was 23 mm while Ciprofloxacin was 16 mm. Garlic contains a variety of oil and water soluble sulfur compounds for which many biological activities of garlic attributed[28]. *Bacillus megaterium* was the most sensitive bacteria with 26 mm inhibition zone, while *Corynebacterium xerosis* and *Staphylococcus aureus* have 25 mm, *Proteus vulgaris* with 24 mm, *Citrobacter freundii* and *Escherichia coli* with 23 mm, *Pseudomonas aeruginosa* with 21 mm and finally *Streptococcus pyogenes* with inhibition zone of 18 mm.

Previous reports cleared that fresh garlic extract displayed evident inhibition properties against MRSA, yet weak inhibition properties against *P. aeruginosa*. Additionally, fresh garlic extract showed the potential to improve the effect of antibiotics on antibiotic resistant pathogens[29]. The antibacterial activity associated with garlic is due to diallylthiosulfonate which called Allicin that has a broad spectrum of cellular targets and it is effective against bacteria, fungi and protozoa with understood mode of action[30].

CONCLUSION

Fresh juice of *Allium sativum* has strong antibacterial activity against the isolate; hot water extract of *Myrtus communis* has weak antibacterial activity while fresh juice of *Allium cepa* has no antibacterial activity against the isolates.

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Cite this article as:

Miaad Hamzah Zghair *et al*. Bacteriological profile of burn patients and antimicrobial susceptibility pattern of bio film forming isolates toward antibiotic and plant extracts. Int. Res. J. Pharm. 2019; 10(5):27-30 http://dx.doi.org/10.7897/2230-8407.1005157

Source of support: Nil, Conflict of interest: None Declared