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Bacterial isolated from burn wound patients, study resistance to antimicrobials and effect of Kombucha (Khubdat Humza) tea on isolates bacteria

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Abstract

A study was conducted from June to November 2012 at Burn Center of Teaching Al-Yarmuk Hospital (Al-Karkh) and Burn Hall of Teaching Al-Kindy Hospital (Al-Rusafa) in Baghdad City. In this study colorimetric VITEK-2 Compact system was used to identify isolates and to detect susceptibility test to several antimicrobial agents. The study also investigated the antibacterial effect of Kombucha tea on isolated bacteria from burn wound patients. The bacteria isolated were nine gram negative bacteria, included, *Acinetobacter baumannii* 24 (23%), *Enterobacter spp.*4 (4%), *Escherichia coli* 9 (8%), *Klebsiella oxytoca* 6 (6%), *Micrococcus luteus* 3 (3%), *Morganella morganii* 4 (4%), *Proteus mirabilis* 3 (3%), *Pseudomonas aeruginosa* 22 (21%) and *Serratia marcescens* 4 (4%) and four gram positive bacteria: *Enterococcus faecalis* 2 (2%), *Staphylococcus aureus* 15 (14%), *Staphylococcus haemolyticus* 6 (6%) and *Staphylococcus hominis* 2 (2%). The results of antimicrobial susceptibility test against gram negative and positive bacteria showed the majority of isolates were resistant to most antimicrobials. The MIC values ranged from (≤ 0.125 - ≤ 320 µg/ml).

The effect of Kombucha (KH) tea on all isolates was at 7days of incubation, the diameter of inhibition zone was 6mm for *Acinetobacter baumannii*, *Proteus mirabilis* and *Serratia marcescens*. 7mm for *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Micrococcus luteus*, *Staphylococcus aureus, Staphylococcus haemolyticus* and *Streptococcus hominis* 8mm for *Morganella morganii*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. The maximum activity of fermented tea was recorded at 14days incubation of Kombucha (KH) colony against all isolates, the diameter of inhibition zone was 22mm for *Acinetobacter baumannii*, 24mm for *Enterobacter cloacae*, *Micrococcus luteus, Serratia marcescens, Staphylococcus aureus, Staphylococcus haemolyticus and Staphylococcus hominis.* 23mm for *Escherichia coli*, 16mm for *Klebsiella oxytoca*, 25mm for *Morganella morganii* and *Enterococcus faecalis*, 20mm for *Proteus mirabilis* and 26mm for *Pseudomonas aeruginosa*. The antibacterial activity of Kombucha (KH) tea decreased with increased incubation periods (28 days).

Keywords: Burn wound, Vitek-2 compact, Antimicrobials, Kombucha (Khubdat Humza) tea

Introduction

Burn wounds are highly susceptible to infection and this is a major problem in the management of burn victims (Al-Ibran *et al*., 2013). Infected burn wounds are not only associated with a delay in epidermal maturation and deep scar formation (Begum *et al*., 2011). Infected patients also tend to stay longer in the hospital and have a higher mortality rate due to sepsis when compared with non-infected patients (Manikandan and Amsath, 2013). An estimated 75% of all deaths following thermal injuries are related to infections (Idomir *et al*., 2012; Ikpeme *et al*., 2013; Valarmathi *et al*., 2013).

The pathogenesis of colonization, infection and invasion of microorganisms is related to the fact that there is a disruption of the normal skin barrier at the site, as well as a large amount of necrotic tissue and protein-rich wound exudates at the burn surface, providing a rich growth medium for colonization and growth of microorganisms, which is poorly controlled due to depressed immune responses (Kalantar *et al*., 2012).

A variety of organisms have been isolated from burn wound colonization and infections. Aerobic bacterial isolates from burn wounds have ranged from Gram-positive organisms like *Staphylococcus aureus*, coagulase negative Staphylococci and *Enterococcus* spp, to Gram-negative organisms like *Pseudomonas aeruginosa, Escherichia coli,*

Klebsiella pneumoniae, Serratia marcescens, Enterobacter spp, *Proteus* spp and *Acinetobacter* spp (Taya *et al*., 2012).The incidence of infections due to less commonly encountered microbes is increasing, as are multidrug-resistant strains of the more common isolates (Sasirekha, 2013; Bhat, and VinodKumar, 2013). Polyantibiotic resistance has been noted in Gram-positive organisms like methicillin-resistant *S. aureus* (MRSA), and also in Gram-negative bacilli like *P. aeruginosa* and *Acinetobacter* spp (Magnet *et al*., 2013; Taherikalani *et al*., 2013). The bacteriological spectrum and antibiogram of burn wound infections and colonization can vary in different health care settings.

A series of the VITEK systems (BioMeriux, Marcy l'Etoile, France) has been a fully automated instrument that provides species identification (ID) and antimicrobial susceptibility testing (AST) for a variety of clinical isolates, and are presently used in many clinical microbiology laboratories worldwide. During the past 3 decades, several revisions have been introduced to the system, resulting in a stepwise improvement of the system performance. Recently, extensive revisions, including reintroduction of colorimetric reading in lieu of fluorescence technology, and addition of several biochemical substrates and taxa covered by the broadened database comparable with the well-established API series (BioMeriux) are created (Nakasone *et al*., 2007; Shetty *et al*., 1998; Sönksen *et al*., 2010). The efforts have been focused upon the accurate ID, in particular, to solve its inherent weakness in the IDs of glucose-nonfermentative Gram-negative rods (GNR) and members of the family Streptococcaceae (Sellenriek *et al*., 2005).

Kombucha is a symbiotic association of bacteria (*Acetobacter xylinum* and *Bacterium gluconicum*) and yeast strains (*Zygossacchromyces kombuchensis*, *Pichia flu Sellenriek xum* and *Saccharomyces sp*.) (Sreeramulu *et al*., 2000). The variation of its composition could be due to geographic, climatic and cultural conditions as well as diversity of local species of wild yeasts and bacteria (Deghrigue *et al*., 2013). These microorganisms are able to grow in culture medium formed of tea infusions (black, mate and green), supplemented with a carbon source. The broth fermented is called "tea fungus" and is originally from the north-east of China (Manchuria). The beverage was introduced in Russia by oriental merchants and then into Eastern Europe and Europe around the turn of this century. This refreshing beverage tasting like sparkling apple cider is often produced at home by fermentation using a tea fungus passed from house to house (Talawat *et al*., 2006). The fermentation and oxidation processes starts, when the tea fungus is placed in a freshly prepared infusion of tea and sugar. When grown in sucrose medium, colonies of yeast break the sucrose in glucose and fructose, then produce carbon dioxide and ethanol, which oxidize to acetaldehyde by bacteria of the colonies. The tea fungus produces many other substances, like gluconic acid and vitamins, which with the supply of tea nutrients, give the drink its unusual flavor and healing properties. The glucose is polymerized and produces cellulose and hemicellulose (Santos *et al*., 2009; Velićanski *et al*., 2013). A wide range of flavor compounds, including alcohols, aldehydes, ketnes, esters and amino acids have been identified from fermented broth (Wu *et al*., 2013). The fermentation using Kombucha colonies is composed of two portions, a floating cellulose pellicle layer, formed during the fermentation by *A. xylinum*, and the sour liquid broth (fermented broth) (Goh *et al*.,2012). The fermentation using Kombucha as a biological agent is conducted at ambient temperature for up to 7-10 days and produces a carbonated fermented broth, softly acid and with low concentration of ethanol. This broth presents beneficial effects, such as antibiotic properties, regulation of gas-gastric, intestinal and glandular activities, relief of joint rheumatism, gout and hemorrhoids, positive influence on the cholesterol level, arteriosclerosis, toxin excretion and blood cleansing, diabetes, and aging problems, and it has been claimed to be a prophylactic and therapeutic beneficial agent to human health, from weight loss to curing cancer (Santos *et al*., 2009; Dufresne and Farnworth, 2000). The beneficial effects of Kombucha tea are attributed to the presence of tea polyphenols, gluconic acid, glucuronic acid, lactic acid, vitamins, amino acids, antibiotics and a variety of micronutrients produced during the fermentation (Ibrahim, 2013).

In Iraq, zoogleal mats of personally imported Kombucha are individually circulated among those people seeking for health remedy. So, this mat is called "Khubdat Humza" as an acquired local traditional Iraqi name. The present study was carried out to determine the aerobic bacterial burn wound isolates from patients attended to Baghdad hospitals and describes their resistance patterns; the study also investigates the antimicrobial effect of kombucha (Khubdat Humza) tea.

Materials and Methods

Isolation & Identification of Bacteria: Samples from margins and edges of burn wound were collected

with help of two sterile swabs, one for gram stain and one for culture before antiseptic dressing was applied. Then swabs were immediately transported to the laboratory for culture. Samples obtained with swab sticks were streaking onto surface of Nutrient agar, Blood agar and MacConkey agar media. The plates were incubated at 37°C for 24-48hrs. The identification of isolates was based on microscopic morphology, staining characteristics, culture and biochemical properties using ID card (GN card and GP card), Vitek 2 compact BioMeriux Company (Nakasone *et al*., 2007).

Antimicrobial susceptibility test: The antimicrobial susceptibility test was performed using several types of AST card, Vitek 2 compact BioMeriux Company (Shetty *et al*., 1998).

Preparation of Kombucha (KH) Tea

Starter culture of Kombucha (Khubdat Humza) was of unknown origin and was provided by Iraqi citizen. Kombucha was prepared by adding 100g/l (10%) weight/volume sucrose to water that had been just boiling for 15 minutes. Subsequently, black tea (Apple tea, UAE, 0.5% w/v) was added and allowed to steep for 15 minutes and then filtered through a sterile sieve. The tea was then cooled to 25°C, and 400ml of tea was aliquot into a 750ml glass bottle that had been previously sterilized at 121°C for 20 minutes. The tea broth was then inoculated with 5g of freshly grown tea fungus that had been cultured in the same medium for 14 days, and the bottle was covered with sterile tissue paper towels to allow aeration. Fermentation was carried out in a dark incubator at 25°C (Sreeramulu *et al*., 2000).

Antimicrobial Activity of Kombucha (KH) tea: Antimicrobial activity was demonstrated by agar diffusion assay. Mueller Hilton agar medium (20 ml) was poured into each Petri dish (90 mm diameter). Suspensions (100 μl) of target strain cultured for 24 h were spread on the plates uniformly, and wells of 9 mm diameter were made with a sterile cork porer. Kombucha samples were centrifuged at 40000*g* force (Du Pont centrifuge, Sorvall RC-5B) for 15 min to remove cell debris. Sterile supernatant was obtained by filtering the supernatant through a sterile microfilter (Millex-GV filter, 0.22 μm pore size, Millipore). Sterile samples (100 μl) were then transferred into the wells of agar plates inoculated with target strains. The plates were then incubated at 37 °C. The diameter of the inhibition zone was measured after 12-15 h. Fermented tea sample was taking after 0, 7, 14, 21 and 28 days of incubation (Sreeramulu *et al*., 2000).

Results and Discussion

The bacteria isolated from burn wound samples are shown in (Table 1). nine gram negative bacteria, namely, *Acinetobacter baumannii* 24 (23%), *Enterobacter spp.*4 (4%), *Escherichia coli* 9 (8%), *Klebsiella oxytoca* 6 (6%), *Micrococcus luteus* 3 (3%), *Morganella morganii* 4 (4%), *Proteus mirabilis* 3 (3%), *Pseudomonas aeruginosa* 22 (21%) and *Serratia marcescens* 4 (4%) and four gram positive bacteria, *Enterococcus faecalis* 2 (2%), *Staphylococcus aureus* 15 (14%), *Staphylococcus haemolyticus* 6 (6%) and *Staphylococcus hominis* 2 (2%) were isolated from the burn wound samples.

The results of antimicrobial susceptibility test against gram negative bacteria shows the all isolates were resistant to Ampicillin, Amoxicillin/Clavulanic Acid, Cefazolin, Ceftriaxone, Aztreonam, Gentamicin, Tetracycline and Ttimethprim/Sulfamethoxazole. And sensitive to Piperacillin/Tazobactam, Cefepime, Ertapenem, Imipenem, Meropenem, Amikacin, Ciprofloxacin and Levofloxacin (Tables 2-1, 2-2).

The antimicrobial susceptibility test against gram positive bacteria shows all isolates were resistance to Cefoxitin Screen, Benzylpenicillin, Ampicillin, Oxacillin, Gentamicin, Erythromycin, Quinupristin/Dalfopristin, Vancomycin, Tetracycline, Rifampicin and Trimethoprim/ Sulfamethoxazole. and sensitive to Gentamicin High Level, Strepmycin High Level, Ciprofloxacin, Levofloxacin, Moxifloxacin, Inducible Clindamycin Resistance, Clindamycin and Tigecycline (Tables 3-1, 3-2).

The result of Minimum Inhibitory Concentration of antimicrobials against gram negative bacteria Isolates shows that the MIC of Ampicillin, Amoxicillin/Clavulanic Acid, Cefazolin, Ceftriaxone, Aztreonam, Gentamicin and Tetracycline were (≤ 16 - ≤ 32 μg/ml), the MIC of Ttimethprim/Sulfamethoxazole was (≤ 160 - ≤ 320 μg/ml), the MIC of Piperacillin/Tazobactam was (≤ 4 - ≤ 8 μg/ml), the MIC of Cefepime was (≤ 1 - ≤ 4 μg/ml), the MIC of Ertapenem and Meropenem were (≤ 0.125 - ≤ 1 μg/ml), the MIC of Imipenem was (≤ 1 - ≤ 2 μg/ml), the MIC of Amikacin was (≤ 2 - ≤ 8 μg/ml), the MIC of Ciprofloxacin was (≤ 0.25 - ≤ 2 μg/ml), the MIC of Levofloxacin was (≤ 0.5 - ≤ 1 μg/ml) as shown in Tables 4-1 and 4-2.

Table (1): Bacteria isolated from burn wounds.

Isolate	No.	Percentage
Acinetobacter baumannii	24	23
Enterobacter cloacae	4	4
Escherichia coli	9	8
Klebsiella oxytoca	6	6
Micrococcus luteus	3	3
Morganella morganii	4	4
Proteus mirabilis	3	3
Pseudomonas aeruginosa	22	21
Serratia marcescens	4	4
Enterococcus faecalis	2	2
Staphylococcus aureus	15	14
Staphylococcus haemolyticus	6	6
Staphylococcus hominis	2	2
Total	104	100

Table (2-1): Susceptibility tests of antimicrobials on gram negative bacteria isolated from burn wounds.

Bacterial isolates	Percentage of resistance							
	AM	AUC	PPL	CZ	CI	CEF	AZ	EPM
Acinetobacter baumannii	88	80	39	81	84	44	77	30
Enterobacter cloacae	90	81	37	83	82	40	75	31
Escherichia coli	91	85	45	82	81	42	74	33
Klebsiella oxytoca	87	83	40	88	85	43	76	36
Micrococcus luteus	90	81	41	80	90	44	77	31
Morganella morganii	85	77	38	82	86	46	71	35
Proteus mirabilis	87	79	39	83	94	47	73	37
Pseudomonas aeruginosa	84	81	40	84	81	41	77	31
Serratia marcescens	86	76	40	81	81	42	74	33

AM= Ampicillin, AUC= Amoxicillin/Clavulanic Acid, PPL= Piperacillin/Tazobactam, CZ= Cefazolin, CI= Ceftriaxone, CEF= Cefepime, AZ= Aztreonam, EPM= Ertapenem.

Table (2-2): Susceptibility tests of antimicrobials on gram negative bacteria isolated from burn wounds.

Bacterial isolates	Percentage of resistance							
	IPM	MPM	AK	GM	CIP	LEV	T	TRI
Acinetobacter baumannii	37	33	40	70	33	39	80	88
Enterobacter cloacae	33	34	39	72	30	37	88	85
Escherichia coli	39	32	41	73	34	33	82	80
Klebsiella oxytoca	33	35	40	77	36	31	85	80
Micrococcus luteus	35	34	39	74	33	38	80	84
Morganella morganii	38	33	40	72	38	36	83	82
Proteus mirabilis	37	37	38	75	31	36	82	88
Pseudomonas aeruginosa	39	30	33	70	38	38	80	87
Serratia marcescens	33	37	36	75	35	32	84	85

IPM= Imipenem, MPM= Meropenem, AK= Amikacin, GM= Gentamicin, CIP= Ciprofloxacin, LEV= Levofloxacin, T= Tetracycline, TRI= Ttimethprim/Sulfamethoxazole.

Table (3-1): Susceptibility tests of antimicrobials on gram positive bacteria isolated from burn wounds.

Bacterial isolates	Percentage of resistance									
	CEF	BEP	AM	OX	GM HL	S HL	GM	CIP	LEV	MOF
Enterococcus faecalis	67	63	68	70	30	33	66	30	31	32
Staphylococcus aureus	77	73	78	80	38	37	76	33	35	33
Staphylococcus haemolyticus	74	77	76	84	39	33	74	30	32	32
Staphylococcus hominis	76	75	79	83	37	35	77	31	33	34

CEF= Cefoxitin Screen, BEP= Benzylpenicillin, AM= Ampicillin, OX= Oxacillin, GMHL= Gentamicin High Level, SHL= Strepmycin High Level, GM= Gentamicin, CIP= Ciprofloxacin, LEV=Levofloxacin, MOF= Moxifloxacin.

Table (3-2): Susceptibility tests of antimicrobials on gram positive bacteria isolated from burn wounds.

Bacterial isolates	Percentage of resistance									
	I CLM R	E	CLM	QUP	V	T	TIG	F	RIP	TRI
Enterococcus faecalis	33	80	40	78	57	75	41	87	54	67
Staphylococcus aureus	37	90	44	88	55	81	43	90	55	77
Staphylococcus haemolyticus	38	87	43	85	56	80	45	91	70	74
Staphylococcus hominis	33	88	45	87	60	79	47	92	71	70

ICLMR=Inducible Clindamycin Resistance, E= Erythromycin, CLM= Clindamycin, QUP=Quinupristin/Dalfopristin, V= Vancomycin, T= Tetracycline, TIG= Tigecycline, F= Nitrofurantoin, RIP= Rifampicin, TRI= Trimethoprim/Sulfamethoxazole.

Table (4-1): Minimum Inhibitory Concentration of antimicrobials against gram negative bacteria Isolates from burn wounds.

Bacterial isolates	MIC (µg/l)							
	AM	AUC	PPL	CZ	CI	CEF	AZ	EPM
Acinetobacter baumannii	≤16 - ≤ 32	≤16 - ≤ 32	≤4 - ≤ 8	≤32- ≤ 64	≤32- ≤ 64	≤1 - ≤ 4	≤16 - ≤ 32	≤0.125- ≤ 1
Enterobacter cloacae	≤16 - ≤ 32	≤16 - ≤ 32	≤4 - ≤ 8	≤32- ≤ 64	≤32- ≤ 64	≤1 - ≤ 4	≤16 - ≤ 32	≤0.125- ≤ 1
Escherichia coli	≤16 - ≤ 32	≤16 - ≤ 32	≤4 - ≤ 8	≤32- ≤ 64	≤32- ≤ 64	≤1 - ≤ 4	≤16 - ≤ 32	≤0.125- ≤ 1
Klebsiella oxytoca	≤16 - ≤ 32	≤16 - ≤ 32	≤4 - ≤ 8	≤32- ≤ 64	≤32- ≤ 64	≤1 - ≤ 4	≤16 - ≤ 32	≤0.125- ≤ 1
Micrococcus luteus	≤16 - ≤ 32	≤16 - ≤ 32	≤4 - ≤ 8	≤32- ≤ 64	≤32- ≤ 64	≤1 - ≤ 4	≤16 - ≤ 32	≤0.125- ≤ 1
Morganella morganii	≤16 - ≤ 32	≤16 - ≤ 32	≤4 - ≤ 8	≤32- ≤ 64	≤32- ≤ 64	≤1 - ≤ 4	≤16 - ≤ 32	≤0.125- ≤ 1
Proteus mirabilis	≤16 - ≤ 32	≤16 - ≤ 32	≤4 - ≤ 8	≤32- ≤ 64	≤32- ≤ 64	≤1 - ≤ 4	≤16 - ≤ 32	≤0.125- ≤ 1
Pseudomonas aeruginosa	≤16 - ≤ 32	≤16 - ≤ 32	≤4 - ≤ 8	≤32- ≤ 64	≤32- ≤ 64	≤1 - ≤ 4	≤16 - ≤ 32	≤0.125- ≤ 1
Serratia marcescens	≤16 - ≤ 32	≤16 - ≤ 32	≤4 - ≤ 8	≤32- ≤ 64	≤32- ≤ 64	≤1 - ≤ 4	≤16 - ≤ 32	≤0.125- ≤ 1

AM= Ampicillin, AUC= Amoxicillin/Clavulanic Acid, PPL= Piperacillin/Tazobactam, CZ= Cefazolin, CI= Ceftriaxone, CEF= Cefepime, AZ= Aztreonam, EPM= Ertapenem.

Table (4-2): Minimum Inhibitory Concentration of antimicrobials against gram negative bacteria Isolates from burn wounds.

Bacterial isolates	MIC (µg/l)							
	IPM	MPM	AK	GM	CIP	LEV	T	TRI
Acinetobacter baumannii	≤1- ≤ 2	≤0.125 - ≤ 1	≤2 - ≤ 8	≤16 - ≤ 32	≤0.25 - ≤ 2	≤0.5 - ≤ 1	≤16 - ≤ 32	≤160 - ≤ 320
Enterobacter cloacae	≤1- ≤ 2	≤0.125 - ≤ 1	≤2 - ≤ 8	≤16 - ≤ 32	≤0.25 - ≤ 2	≤0.5 - ≤ 1	≤16 - ≤ 32	≤160 - ≤ 320
Escherichia coli	≤1- ≤ 2	≤0.125 - ≤ 1	≤2 - ≤ 8	≤16 - ≤ 32	≤0.25 - ≤ 2	≤0.5 - ≤ 1	≤16 - ≤ 32	≤160 - ≤ 320
Klebsiella oxytoca	≤1- ≤ 2	≤0.125 - ≤ 1	≤2 - ≤ 8	≤16 - ≤ 32	≤0.25 - ≤ 2	≤0.5 - ≤ 1	≤16 - ≤ 32	≤160 - ≤ 320
Micrococcus luteus	≤1- ≤ 2	≤0.125 - ≤ 1	≤2 - ≤ 8	≤16 - ≤ 32	≤0.25 - ≤ 2	≤0.5 - ≤ 1	≤16 - ≤ 32	≤160 - ≤ 320
Morganella morganii	≤1- ≤ 2	≤0.125 - ≤ 1	≤2 - ≤ 8	≤16 - ≤ 32	≤0.25 - ≤ 2	≤0.5 - ≤ 1	≤16 - ≤ 32	≤160 - ≤ 320
Proteus mirabilis	≤1- ≤ 2	≤0.125 - ≤ 1	≤2 - ≤ 8	≤16 - ≤ 32	≤0.25 - ≤ 2	≤0.5 - ≤ 1	≤16 - ≤ 32	≤160 - ≤ 320
Pseudomonas aeruginosa	≤1- ≤ 2	≤0.125 - ≤ 1	≤2 - ≤ 8	≤16 - ≤ 32	≤0.25 - ≤ 2	≤0.5 - ≤ 1	≤16 - ≤ 32	≤160 - ≤ 320
Serratia marcescens	≤1- ≤ 2	≤0.125 - ≤ 1	≤2 - ≤ 8	≤16 - ≤ 32	≤0.25 - ≤ 2	≤0.5 - ≤ 1	≤16 - ≤ 32	≤160 - ≤ 320

IPM= Imipenem, MPM= Meropenem, AK= Amikacin, GM= Gentamicin, CIP= Ciprofloxacin, LEV= Levofloxacin, T= Tetracycline, TRI= Ttimethprim/Sulfamethoxazole.

The result of Minimum Inhibitory Concentration of antimicrobials against gram positive bacteria Isolates shows that the MIC of Cefoxitin Screen was (≤ 8 - ≤ 16 µg/ml), the MIC of Benzylpenicillin, Ampicillin, Oxacillin, Gentamicin, Quinupristin/Dalfopristin, Vancomycin and Rifampicin were (≤ 16 - ≤ 32 µg/ml), the MIC of Gentamicin High Level, Strepmycin High Level and Levofloxacin were (≤ 1- ≤ 4 µg/ml), the MIC of Ciprofloxacin was (≤ 0.5 - ≤ 1 µg/ml), the MIC of Moxifloxacin was (≤ 0.125 - ≤ 1 µg/ml), the MIC of Inducible Clindamycin Resistance was (≤ 0.5 - ≤ 2 µg/ml), the MIC of Erythromycin and Tetracycline were (≤ 8 - ≤ 16 µg/ml), the MIC of Clindamycin and Tigecycline were (≤ 0.25 - ≤ 1 µg/ml), the MIC of Nitrofurantoin was (≤ 64- ≤ 256 µg/ml), the MIC of Trimethoprim/Sulfamethoxazole was (≤ 160 - ≤ 320 µg/ml) Tables 5-1 and 5-2.

The data in (Table 6) shows that Kombucha (KH) tea has effective antibacterial activities on the burn wounds isolates as indicated by the diameter of their zone of inhibition. The effect of Kombucha (KH) tea on all isolates was at 7days of incubation, the diameter of inhibition zone was 6mm for *Acinetobacter baumannii*, *Proteus mirabilis* and *Serratia marcescens*. 7mm for *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Micrococcus luteus*, *Staphylococcus aureus, Staphylococcus haemolyticus* and *Streptococcus hominis* 8mm for *Morganella morganii*, *Pseudomonas aeruginosa* and *Enterococcus faecalis*. The maximum activity of fermented tea was recorded at 14days incubation of Kombucha (KH) colony against all isolates, the diameter of inhibition zone was 22mm for *Acinetobacter baumannii*, 24mm for *Enterobacter cloacae*, *Micrococcus luteus, Serratia marcescens, Staphylococcus aureus, Staphylococcus haemolyticus and Staphylococcus hominis.* 23mm for *Escherichia coli*, 16mm for *Klebsiella oxytoca*, 25mm for *Morganella morganii* and *Enterococcus faecalis*, 20mm for *Proteus mirabilis* and 26mm for *Pseudomonas aeruginosa*. The antibacterial activity of Kombucha (KH) tea decrease with increase incubation periods (28 days).

Several species of bacteria were isolated from patients with various degrees of burn wound infections which include, *Acinetobacter baumannii*, *Enterobacter cloacae*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella oxytoca*, *Micrococcus luteus*, *Morganella morganii*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, *Staphylococcus haemolyticus* and *Staphylococcus hominis*. Similar organisms have been reported (Al-Ibran *et al*., 2013; Begum *et al*., 2011; Manikandan and Amsath, 2013; Idomir *et al*., 2012; Ikpeme *et al*., 2013; Valarmathi *et al*., 2013; Kalantar *et al*., 2012; and Taya *et al*., 2012).

Overall, the evaluation results of the newly redesigned colorimetric VITEK-2 ID was so impressed by the performance because more than 98% of the isolates were correctly identified to the species level without any further additional tests.

Table (5-1): Minimum Inhibitory Concentration of antimicrobials against gram positive bacteria Isolates from burn wounds.

I. No.	MIC (µg/l)									
	CEF	BEP	AM	OX	GM HL	S HL	GM	CIP	LEV	MOF
1	≤8 - ≤ 16	≤1 - ≤ 8	≤16 - ≤ 32	≤4 - ≤ 16	≤1 - ≤ 4	≤1 - ≤ 4	≤16 - ≤ 32	≤0.5- ≤1	≤1- ≤4	≤.125- ≤1
2	≤8 - ≤ 16	≤1 - ≤ 8	≤16 - ≤ 32	≤4 - ≤ 16	≤1 - ≤ 4	≤1 - ≤ 4	≤16 - ≤ 32	≤0.5- ≤1	≤1- ≤4	≤.125- ≤1
3	≤4 - ≤ 8	≤1 - ≤ 8	≤8 - ≤ 16	≤8 - ≤ 16	≤1 - ≤ 4	≤1 - ≤ 4	≤16 - ≤ 32	≤0.5- ≤1	≤1- ≤4	≤.125- ≤1
4	≤8 - ≤ 16	≤1- ≤ 4	≤8 - ≤ 16	≤8 - ≤ 16	≤1 - ≤ 4	≤1 - ≤ 4	≤16 - ≤ 32	≤0.5- ≤1	≤1- ≤4	≤.125- ≤1

1= *Enterococcus faecalis,* 2= *Staphylococcus aureus*, 3= *Staphylococcus haemolyticus*, 4= *Staphylococcus hominis*. I. No. = Isolate Number.
CEF= Cefoxitin Screen, BEP= Benzylpenicillin, AM= Ampicillin, OX= Oxacillin, GMHl= Gentamicin High Level, SHL= Strepmycin High Level, GM= Gentamicin, CIP= Ciprofloxacin, LEV=Levofloxacin, MOF= Moxifloxacin.

Table (5-2): Minimum Inhibitory Concentration of antimicrobials against gram positive bacteria Isolates from burn wounds.

I. No.	MIC (µg/l)									
	ICLMR	E	CLM	QUP	V	T	TIG	F	RIP	TRI
1	≤0.5 - ≤ 2	≤8 - ≤ 16	≤0.25 - ≤ 1	≤16 - ≤ 32	≤0.5 - ≤ 1	≤8 - ≤ 16	≤0.25 - ≤ 1	≤64 - ≤ 256	≤0.5- ≤ 1	≤160 - ≤ 320
2	≤0.5 - ≤ 2	≤8 - ≤ 16	≤0.25 - ≤ 1	≤16 - ≤ 32	≤0.5 - ≤ 1	≤8 - ≤ 16	≤0.25 - ≤ 1	≤64 - ≤ 256	≤0.5- ≤ 1	≤160 - ≤ 320
3	≤0.5 - ≤ 2	≤8 - ≤ 16	≤0.25 - ≤ 1	≤16 - ≤ 32	≤16 - ≤ 32	≤8 - ≤ 16	≤0.25 - ≤ 1	≤64 - ≤ 256	≤16- ≤ 32	≤160 - ≤ 320
4	≤0.5 - ≤ 2	≤8 - ≤ 16	≤0.25 - ≤ 1	≤16 - ≤ 32	≤16 - ≤ 32	≤8 - ≤ 16	≤0.25 - ≤ 1	≤64 - ≤ 256	≤16- ≤ 32	≤160 - ≤ 320

1= *Enterococcus faecalis,* 2= *Staphylococcus aureus*, 3= *Staphylococcus haemolyticus*, 4= *Staphylococcus hominis*. I. No. = Isolate Number.
ICLMR=Inducible Clindamycin Resistance, E= Erythromycin, CLM= Clindamycin, QUP= Quinupristin/Dalfopristin, V= Vancomycin, T= Tetracycline, TIG= Tigecycline, F= Nitrofurantoin, RIP= Rifampicin, TRI= Trimethoprim/Sulfamethoxazole.

Table (6): Antimicrobial activities of Kombucha (KH) tea on bacteria isolated from burn wounds.

Bacterial isolates	Incubation periods of Kombucha colonies				
	0day I.Z(mm)	7days I.Z.(mm)	14days I.Z.(mm)	21days I.Z.(mm)	28days I.Z.(mm)
Acinetobacter baumannii	0.0	6.0	22.0	20.0	10.0
Enterobacter cloacae	0.0	7.0	24.0	21.0	11.0
Escherichia coli	0.0	7.0	23.0	21.0	11.0
Klebsiella oxytoca	0.0	7.0	16.0	14.0	10.0
Micrococcus luteus	0.0	7.0	24.0	20.0	11.0
Morganella morganii	0.0	8.0	25.0	20.0	13.0
Proteus mirabilis	0.0	6.0	20.0	15.0	9.0
Pseudomonas aeruginosa	0.0	8.0	26.0	22.0	13.0
Serratia marcescens	0.0	6.0	24.0	20.0	10.0
Enterococcus faecalis	0.0	8.0	25.0	22.0	13.0
Staphylococcus aureus	0.0	7.0	24.0	20.0	12.0
Staphylococcus haemolyticus	0.0	7.0	24.0	20.0	12.0
Staphylococcus hominis	0.0	7.0	24.0	20.0	12.0

I.Z. = Inhibition Zone.

Also, the present results indicated that the current VITEK-2 has overcome its inherent weakness in IDs of streptococci and glucose-nonfermentative GNR. Until present, API test strips has been long considered as the gold standard Q in ID test (Nakasone *et al*., 2007; Shetty *et al*., 1998). But the accuracy of the VITEK-2 was finally estimated to be 98.3%, compared with 97.5% by the respective API test strips. Our results were highly consistent with a series of evaluation results recently published for GPC (Sellenriek *et al*., 2005), GNR (Sönksen *et al*., 2010).

The antimicrobial activities of commercially prepared antibiotics on the bacterial isolates showed, that all isolates were sensitive to quinolones (Ciprofloxacin, Levofloxacin and Ofloxacin) this agree with many references which showed that most bacteria isolated from burn wounds were sensitive to quinolones compounds (Bhat and VinodKumar, 2013; Magnet *et al*., 2013). The resistance to other types of antimicrobials differs with different isolates; these are in agreement with (Taherikalani *et al*., 2013). The interpretation of results due to littleness using of Quinolones in Baghdad hospitals compared with other antimicrobials such as Ampicillin, Chloramphenicol, Erythromycin, Gentamicin and Oxacillin.

The result of this study shows that Kombucha (KH) tea has antimicrobial actions against all bacteria isolated from burn wound infections. Table (3) shows that all the isolates were sensitive to Kombucha tea even at 7 days of incubation. The interpretation of these results may be due to decreased pH of the Kombucha tea with subsequent fermentation time. During the fermentation process, yeasts and bacteria metabolize sucrose into a number of organic acids, such as acetic acid and gluconic acid. Therefore due to an increased concentration of these organic acids, the pH decreased from 5 to 2.5 within 6 days of fermentation and remained stable thereafter. These observations are in agreement with the findings of other studies (Sreeramulu and Knol, 2000; Deghrigue *et al*., 2013). The inhibition of bacterial growth caused by acid shock (low pH).

The maximum antimicrobial effect of Kombucha tea noted at 14 days of incubation table (3), in this period the largest inhibition zones were recorded, this agree with several references (Talawat *et al*., 2006; Santos *et al*., 2009; and Velićanski *et al*., 2013) which found a slight secondary growth of bacteria found in Kombucha tea observed after 12 days of fermentation, likely this is due to multiplication of acid-tolerant bacterial strains therefore the produce of inhibine increases in this period. Results also showed that the antibacterial activity of Kombucha tea decrease with the increasing incubation periods (28 days) this agree with several references (Wu *et al*., 2013; Goh *et al*., 2012) in which interpretation of the results was due to littleness of Carbone source and other nutrients required for Kombucha growth.

The antimicrobial activity of Kombucha under different incubation periods studied against a number of pathogenic microorganisms which causes burn wound infections, Kombucha had its strongest antimicrobial effects, and this implies the existence of an antimicrobial component other than acetic acid and large proteins. There are numerous reports indicating that the polyphenols/ tannins extracted from tea inhibit a broad spectrum of Gram-positive and Gram-negative bacteria. Among the catechins tested, epigallocatechin, epicatechin gallate, and epigallocatechin gallate have been found to be inhibitory for the growth of *S. aureus* and *V. cholerae* (Sreeramulu *et al*., 2000). Other studies (Velićanski *et al*., 2013; Wu *et al*., 2013; and Goh *et al*., 2012) reported that the extracts of green and black tea can inhibit *Cm. jejuni*, *E. coli*, and *H. pylori*. Recently, (Santos *et al*., 2009) have tested the antimicrobial activity of Kombucha as well as normal tea extracts prepared at different concentrations and found that the inhibitory effects of Kombucha increased with the tea concentration. In our studies, the concentration of tea broth was 0.5% for the preparation of Kombucha. The polyphenol/ tannin level in such low concentration of tea was unlikely to have an inhibitory effect against the target microorganisms. Hence, these findings suggest the presence of an antimicrobial compound other than acetic acid, large proteins, and catechins in Kombucha. Antimicrobial activity increased with fermentation time until 21days (Dufresne and Farnworth, 2000). As seen in almost all cases tested. This also implies that the active antimicrobial components are very likely metabolites produced by the bacteria and/or yeasts responsible for the fermentation of Kombucha .At present a characterization of antimicrobial compounds is in progress (Ibrahim, 2013).

Conclusions

The present study concluded that Kombucha (KH) tea is the best alternative medicine material has very good antimicrobial activity against pathogenic bacteria. We recommend further studies on the possibility on using Kombucha (KH) scoby as biological agent in treatment of burn wounds. This agent has two important characteristics, antimicrobial activity, and used for medical

purposes in skin therapy. The cellulosic pellicle formed mainly by *Acetobacter xylinum* during the fermentation of tea has been used as a temporary skin substitute on burns and in other skin injuries (Barud *et al*., 2013).

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