

Isolation and Identification of Bacteria from Burn injuries by 16S rRNA Gene.

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Abstract

Burn injuries are common and major health problem throughout the world. The burn wound represents as a favorable area for opportunistic colonization of microorganisms with exogenous and endogenous origin. In burns patients infections arise from multiple sources. Burn wounds become initially colonized bacteria, mainly *Staphylococci*, that are superseded during the second week by Gram negative bacteria. this study was aimed Isolation and identification of bacteria from burn injuries by 16S rRNA Gene . Materials A total of 107 wound samples Who admitted to referral of Hussein Teaching Hospital of the period from 2016 to 2015. All the samples were cultured on medias MacConkey agar , Blood agar, Nutrient agar and mannitol salt agar for the isolation of Gram negative bacteria and Gram positive bacteria. After confirmation through colony characteristics and biochemical tests, API20 and Viteck2, as well as to molecular diagnostics using 16S rRNA gene. all the isolates were evaluated against commonly used antibiotics, for their susceptibility using Viteck system. As for the screening test sensitivity was shown the results of the current study that the most effective antibiotics against both Gram-negative bacilli bacteria and Gram- positive cocci were Ceftazidin, Ciprofloxacin, Amicacin while most of isolates showed high resistance to Ticarkcillin, Gentamicin, Impenen.

Key words: 16S rRNA, clinical pathogens Burn infection.

عزل وتشخيص البكتريا المرافقة لإصابات الحروق بواسطة جين 16SrRNA

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الخلاصة

تعد اصابات الحروق مشكلة صحية شائعة ورئيسية في جميع انحاء العالم وتمثل منطقة او مكان الحرق كمنطقة ملائمة لاستعمار الكائنات الحية الدقيقة الانتهازية سواء كانت خارجية او داخلية المنشأ. في الحروق تنشأ العدوى من مصادر مختلفة ومتعددة , يستوطن الحرق في البداية بالبكتريا الموجبة لصبغة كرام والمتمثلة بصورة رئيسية ببكتريا *Staphylococci* وخلال الاسبوع الثاني تحل الجراثيم السالبة لصبغة كرام. تهدف هذه الدراسة الى تشخيص الانواع البكتيرية المرافقة لاصابات الحروق بواسطة جين 16S rRNA. بلغ العدد الكلي للعينات 107 عينة , زرعت هذه العينات على اوساط اكار الماكونكي , اكار الدم , والاكار المغذي واكار المانيتول للبكتريا السالبة والموجبة لصبغة كرام بعد ذلك تم التشخيص الاولي لهذه العينات باستخدام الخصائص المظهرية والاختبارات البايوكيميائية واجري التشخيص التاكيدي باستخدام نظام API20 ونظام الفايترك Viteck فضلا عن التشخيص الجزيئي باستخدام جين 16SrRNA. تم اجراء فحص الحساسية لجميع العزلات تجاه المضادات الحيوية الشائعة باستخدام نظام الفايترك Viteck. بينت نتائج الدراسة الحالية ان اكثر المضاد الحيوي فعالية ضد كل من البكتريا السالبة والموجبة لصبغة كرام هي Ceftazidin, Ciprofloxacin, Amicacin بينما ابدت كل العزلات مقاومة عالية الى Ticarcillin, Gentamicin, Impenem.

الكلمات المفتاحية : جين, 16SrRNA اصابات الحروق , مسببات الامراض السريرية

Introduction

Use of broad-rang 16S rRNA gene PCR as a tool for identification of bacteria is possible because the 16S rRNA gene is present in all bacteria. The 16S rRNA gene consist of highly conserved nucleotide Sequences interspersed with variable regions that are genus- or species. PCR primers targeting the conserved regions of rRNA amplify variable sequences of the rRNA gene. Bacteria can be identified by nucleotide sequence analysis of the PCR product followed by comparison of this sequence with Known sequences stored in a data base (Claire et al., 2012).

Burn is one of the common incidents that hurts skin and create different types of wound infections. In addition to loss of the natural cutaneous barrier to nutrients in the burn wound lead to microbial colonization. Infection is the major complication in burns and it has been estimated that about 75% of the mortality associated with burn injuries is related to infection (Heimbach, 1999). The wounds can become infected with different types of the organisms and these are increasing in their resistance to antibiotics. The treatment of these infection is one of the most difficult processes to helper covery of patients (Deitch, 1990). Understanding the epidemiology of the infecting organisms in these patients with burn wound infection is important because of immunosuppression and resultant severe complications such as septicemia and pneumonia (Sullivan and Connor et al., 1997; Pruitt, 1998)

Burn injuries remain a major cause of morbidity and mortality in low and middle in come countries. During the past two decades the Iraqi population has being struggling to

cope with the impact of wars, sanctions and internal conflicts with poor public services and deteriorating living standards (Nasih, 2010). Burns constitute a major role in mortality and morbidity in the whole world, whether accidental, suicidal or homicidal. Burn injuries are among the most devastating of all injuries and a major global public health crisis. Burns are the fourth most common type of trauma worldwide, following traffic accidents, falls, and interpersonal violence. Approximately 90% of burns occur in low to middle income countries, regions that generally lack the necessary infrastructure to reduce the incidence and severity of burns (Vaghela et al., 2012).

Material and Methods :

This prospective study was conducted on 107 patients admitted in burn unit. A total of 107 surface swabs were taken using standard methods and cultured for growing of the bacteria which were subjected to antibiotic sensitivity testing. Chronic wound for the purpose of this study was defined as any burn wound with skin loss which failed to heal or epithelize naturally within 3 weeks from the date of injury.

The required data of burn patients including age, sex, season, causes of burns, burn size, manner (way) of burning based on intentional (on purpose) or unintentional (casually). Specimens were cultured on appropriated culture media including MacConkey agar, Blood agar, Nutrient agar and Eosin Methylene Blue Agar. The cultures were incubated in 37°C for 24-48 h and then the colonies were removed for further study. Biochemical and Culture characterization of the isolates were verified for identification purposes (Koneman et al, 1997). In essential cases, specific bacteria was used for precise identification of bacteria type. In order to detect the susceptibility of isolates to antimicrobial drugs, all isolates were tested by Vitek system (Biomérieux company) 8 antibiotics including Ciprofloxacin, Amikacin, Imipenem, Gentamicin, Ticarcillin, Tobramycin, Trimethoprim, Ceftazidim.

Results

During the present study, 107 patients with suspected burn wound infections had their wounds sampled twice. A total of 117 pathogenic and opportunistic bacteria were identified. Single isolates were found in 82.46% of specimens and 8.54% of specimens also yielded double. In all, 109 isolates (93.1%) were Gram-negative bacilli and 8 (6.83%)

were Gram- positive cocci. The predominant bacteria isolated from the infected wounds was *Pseudomonas aeruginosa* (32.47%) followed by *Klabseilla pneumoniae* (21.36%), *Acinetobacter baumannii* (12.82%), *Eschreish coli* (11.1%) , *Enterobacter cloacae* (5.12%), *Staphylococcus aureus* (5.12%), *Burkhoideria cepesa* (4.27%), *Protus miribilla* (3.41%), *Pantoea agglomerans* (2.56%) *Staphylococcus epidermis* (1.7%). (Table 1).

Out of 107 infected patients, 42.9% were males and 57% were females. (Figure 1). As it is shown in Table 2, boiled water (%38.3), Fire by gas flames (28.9%), Fire by gasoline flames(19.6%), Hot liquids (8.41%) and Electrical shock (4.67%) (Table 2). Frequency of the manners of burning based on intention or accidentally was as follow: (86.9%) (93 cases) of patients were burned accidentally and (13%) (14 cases) were intention. (Figure 2).

The most frequent patients were belonged to the 0-9 years old (54.2%) (Table 3). The present results showed that the most frequent burning (75.7%) was occurred during winter, The lowest frequency (24.2%) was belonged to summer. So the most common time of burning of present was during winter. (Figure 3). study found that the highest proportion of cases were among individuals who live in country increased by 90 injured 84.1% while the injuries the city has reached 17 cases of burn injury by 15.88.(Figure 4).

As it is observed from results ,the most effective antibiotics against both Gram-negativ bacilli and Gram-positiv cocci were Ciprofloxacin ,Ceftazidine, Amikacin and Gentamicin while most of isolates showed high resistance to Ampicillin, Tetracycline, and Carbenicillin. All *P. aeruginosa* isolates from wound infection were resistant to Ampicillin. Bacteria 16S rRNA wsa amplified in 50 out of 107 of the sample analysed using the 1500 bp PCR protocol for *P.aeruginosa*, *K.pneumonia*,*A.baumannii*, *E.coli*, *E.claoca*, *S.aureus*,*B.cepase*, *P.miribilla*, *P.agglomerans*, *S.epedermis* .A single discrete PCR amplicon band of 1500bp of 16SrRNA was observed when resolved on Agarose.

Table 1: Frequency distribution of isolated microorganisms from burn.

Isolates	Frequency of isolates	Percentage
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<i>Pseudomonas aerogenosa</i>	38	32.47%
<i>Klabseilla pneumonia</i>	25	21.36%
<i>Acinetobacter baumannii</i>	15	12.82%
<i>Eschresch coli</i>	13	11.1%
<i>Enterobacter cloacae</i>	6	5.12%
<i>Staphylococcus aureua</i>	6	5.12%
<i>Burkholderia cepeas</i>	5	4.27%
<i>Proteus mirabilis</i>	4	3.41%
<i>Pantoea agglomerans</i>	3	2.56%
<i>Staphylococcus epidermis</i>	2	1.7

2:The

Table
frequency

distribution of the causes of burns in patients.

Causes of burn	Frequency	Percentage
Boiled water	41	38.3%
Fire by gas flim	31	28.9%
Fire by gasoline flim	21	19.6%
Hot liquids	9	8.41%

Electrical shock	5	4.67%
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Table 3: The frequency distribution of age groups of burned patients

Age groups	Frequency	percentage
0-9	58	54.2%
10-19	12	11.21%
20-29	22	20.56%
30-39	10	9.34%
40-49	5	4.67%

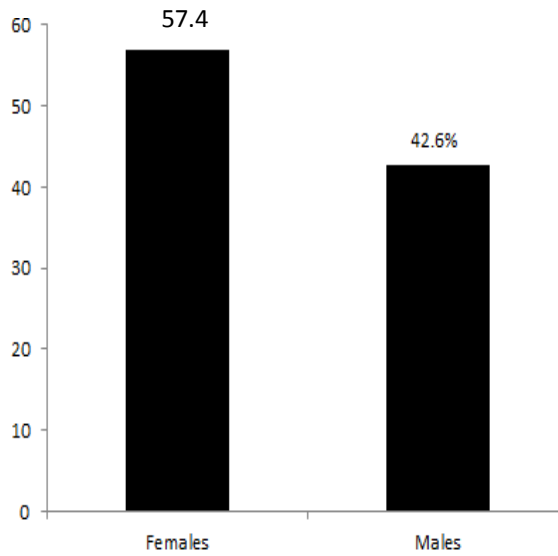


Figure 1: The distribution of the injured according to the gender

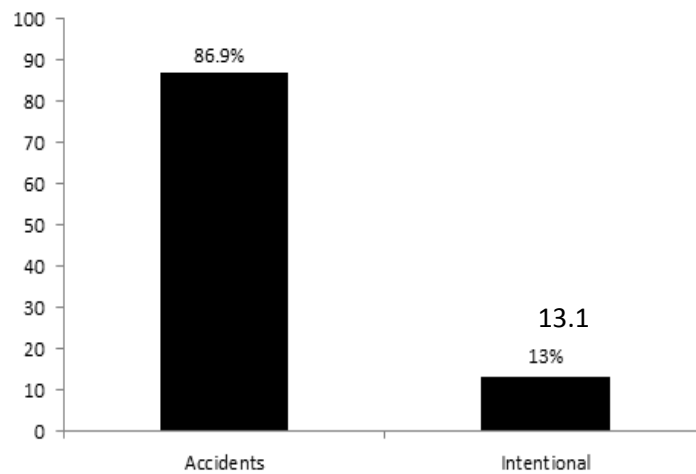


Figure 2: The distribution of the injured accident and intentional

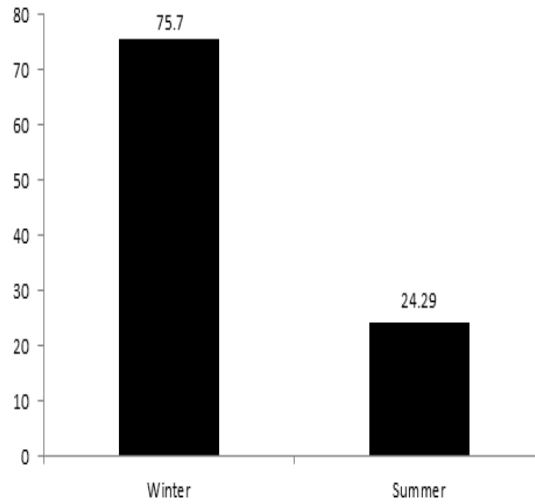


Figure 3: The distribution of the injured according to the seasons

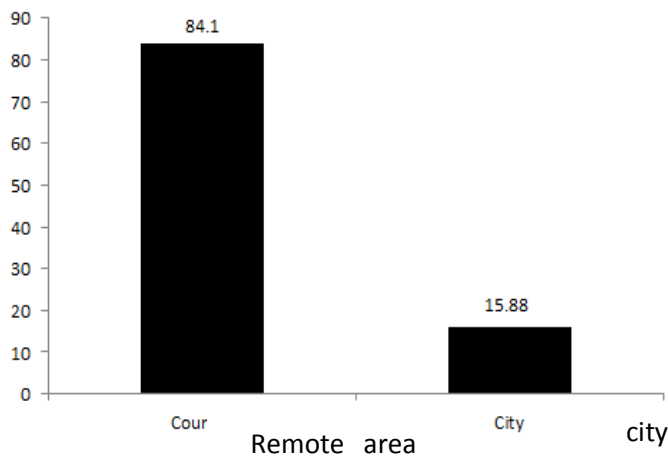


Figure 4: The distribution of the injured according location

M 1 2 3 4 5 6 7 8

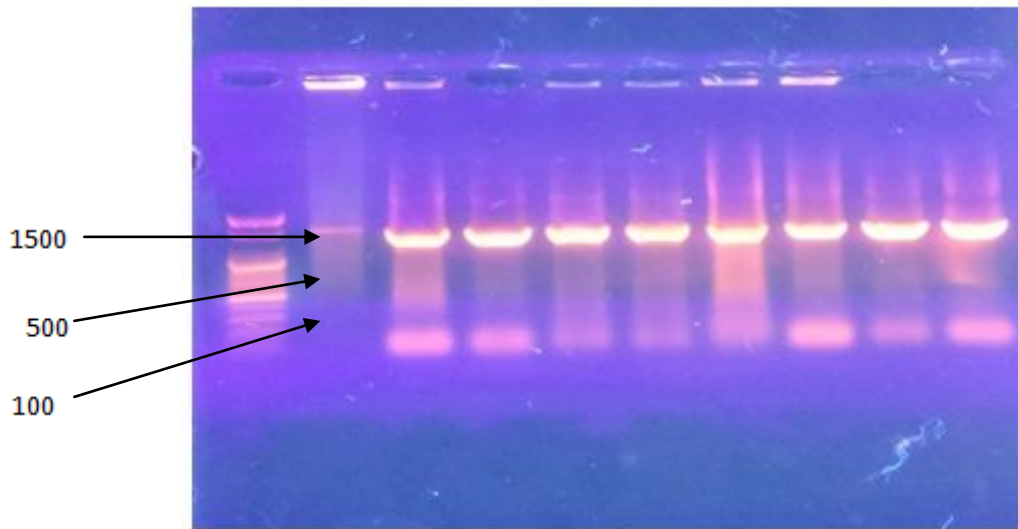


Figure 5 The electrophoresis of PCR product of bacterial isolates using primers quality 16SrRNA gene on agarose concentration 1% Lane:M (Ladder 2000 bp DNA Lane1-8 16SrRNA amplicon of 1: E.cloaca,2 : P.aeruginosa,3:S.aureus,4:S.epidermsis:,5:K.peumonia:6,E.coli: 7, A.baumannii,:8B.cepesa.

Discussion

In the current study results among the 107 patients, (42.9%) were male and (57%) were female. In a study performed in Yemen in 2011, (58%) of patients were femals and (42%) were males. Similar results were obtained by Karimi *et al*, in (2002) were reported that burn wound infection in females were 189 (62.4%) while burn wound infection in males were 114 (37.6%). In a similar study by Church *et al* in (2006) males were more affected Peck (2011) found that (64.2%) of burn infection patients were femals while (35.8%) were males. other studies also had a femal. contrast to kanagapriya *et al*. (2015) showed that burn infection in male 54% (46%) for femle in India.

The Present results showed that children under10 years old were the most age groups of burning victims with 54.2%. Since most of these children are accidentally exposed to burns factors, they needed to be look after carefully. In a spective survey, the effects of several factors on the time of being hospitalized of patients with extensive burning (at least 24% of body) have been investigated (Manson *et al* ., 1992). The length of being in

hospital, has had direct relationship with burn size and patient age. Most wound infections have been created by Enterobacteriaceae or accompanied with *Pseudomonas* species.

The other finding of this study was the causes of burns, the most common causes of burning of patients were boiled water (38.3%) followed by fuel oil flames and firewood flames. Most of victims (86.9%) were accidentally burned and 13% cases were intentional. In these conditions, drug resistant organisms such as *P. aeruginosa* and methicillin resistant *S. aureus* (MRSA) are probably transferred to the burn wound surfaces and provide serious cross-infection Agnihorti *et al* ,(2004)

Antibiotic susceptibility patterns of isolates that were isolated from burn infections showed that, the most effective antibiotics were ciprofloxacin, amikacin, ceftazidim, gentamicin. *P. aeruginosa* and *S. aureus* showed high resistance to many antibiotics such as ticarcillin, imipenem, tobramycin and trimethoprim. These two species mentioned above are versatile human pathogens that continue to be an important cause of nosocomial infections especially in burn units. The emergence and spread of multidrug-resistance among species of *P. aeruginosa* and *S. aureus* has become a major concern worldwide and is seriously challenging current treatment strategies (Karimi *et al* .(2002); Tredget *et al*. (1992); Mayhall (2003) Abdullah *et al*. (2010) ; Agnihortri *et al*. (2004), Present results reveal a broad – spectrum resistance of isolates, both Gram - negative and Gram positive to majority of antibiotics most isolates showed high resistance to Ceftazidim, Ticarcillin and Gentamicin. This study showed that performing 16S rRNA PCR assays has the potential to make an important contribution to patient management by detecting the presence of bacterial pathogens in culture-negative clinical samples. detected the bacterial 16S rRNA gene in more samples. This increase was associated with a compromise in the ability of the assay to identify the bacterial to the species level (Baker *et al*., 2003).

Conclusion

Bacterial infections are a serious problem among burn patients and *P. aeruginosa* has emerged as the commonest organism causing infection and is resistant to most of the antibiotics.

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