Isolation and diagnosis of bacteria causing inflammation resulting from fractures of the lower or upper jaw and treated with Olive leaf extract

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Abstract

Background: A particular attention is given to mandibular fractures owing to the diversity of locations, severity of fractures and the accessibility of different treatment modalities. The aim of current study was to investigate the bacteria causing inflammation of the lower and upper jaws. Methods: In the present study, 134 different samples were collected for various fractures during the period from 2018 to 2019. Types of bacteria causing inflammation were isolated in cases of fractures. The laboratories of the Department of Basic Medical Sciences at the Faculty of Dentistry and by using special laboratory techniques for culturing and diagnosis of bacteria represented by the polymerase chain reaction technique and the use of special genes. Results and Conclusion: Five types of inflammatory bacteria were found in the upper and lower jaw fractures, they were as the following; Acinetobacter baumannii (353 blaOXA-51; 30.05%, Pseudomonas aeruginosa (16SrDNA; 36%, Streptococcus pyogenes (pSM19035_003 ORF alpha; 30%, Staphylococcus aureus (ORF28 similar to putative transposase; 17%. The study found that there were significant differences with different bacterial species with infection rates. The effect of olive leaf extract was also observed, there was a significant effect among bacteria with olive leaf extract. Concentrations of 10%, 20%, 30%, 40%, 50%, 60% and 70% were used. There was a significant moral difference.

Keywords: Bacteria, Fractures, Lower jaw, Upper jaw, Olive leaf extract

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Introduction

Mandibular fractures account for the majority of maxillofacial traumatic injuries. A particular attention is given to mandibular fractures owing to the diversity of locations, severity of fractures and the accessibility of different treatment modalities. In addition, infection of jaw fractures may be revealed for the most commonly encountered postoperative obstacle and mandibular fractures are reported to be associated with the elevated rate of infections among other maxillofacial fractures [1]. Moreover, one of the most challenging complications in trauma surgery is infection after fracture fixation (IAFF). The latter may result in permanent functional loss or even amputation of the affected limb in patients who may otherwise be expected to achieve complete, uneventful healing. Over the past decades, IAFF shares many resemblance with PJI, however, there are numerous critical distinctions in many facets including prevention, diagnosis and treatment. Confessedly, extrapolating data from PJI research to IAFF were of value to the trauma surgeons, but we should also be aware of the special challenges
posed by IAFF that may not be reckon for in the PJI literature \[2\]. These localized grouped bacteria are often metabolically quiescent, which makes them complicated to identify and culture. Cultures taken from an open wound at the time of initial fracture fixation do not correlate with a final later infection and should be avoided \[3\]. Clinical examination revealed that common symptoms and signs of mandibular fracture(s) include pain, especially on talking and swallowing, numbness of the lower lip, swelling, trismus and difficulty in moving the jaw. In addition, bone tenderness over fracture site, altered occlusion, loosened teeth and gingival bleeding, mobility of fractured segment and haematoma associated with fracture site, especially sublingual \[4\]. Furthermore, in a displaced fractured mandible, the patient may sometimes regard as they have lost a tooth when in fact they have not; it is because of mobility at the fracture site \[5\].

Antimicrobial drugs have caused a substantial change not only of the treatment of infectious diseases but also of a fate of mankind. Antimicrobial chemotherapy made extraordinary proceed, resulting in the overly optimistic view that infectious diseases would be defeated in the near the time to come. Also, infections with drug-resistant organisms remain a main problem in clinical practice that is difficult to work out \[6\].

Although olive oil is well-known for its flavor and feasible health profit, the leaf and its extracts remain under preliminary research with unknown effects on human health. Olive phenolics are much more concentrated in the leaves contrast with olive fruit or olive oil, as 1450mg total phenolics/100g fresh leaves and 110mg/100g fruit and 23mg/100 ml extra virgin olive oil. Chemical compounds in unprocessed olive leaf are oleuropein and hydroxytyrosol, as well as polyphenols and flavonoids \[7\].

The aim of current study was to investigate the bacteria causing inflammation of the lower and upper jaws.

**Material and Methods**

A study was carried out to investigate the bacteria causing inflammation of the lower jaw and upper jaw. It included 134 different samples which were collected for various fractures during the period 2018 to 2019. Types of bacteria causing inflammation were isolated in cases of fractures. The laboratories of the Department of Basic Medical Sciences at the Faculty of Dentistry and by using special laboratory techniques for culturing and diagnosis of bacteria represented by the polymerase chain reaction technique and the use of special genes.

**Collection of Samples**

The samples were collected by swabs from the lower jaw and upper jaw and then cultured in bacterial culture media; each isolate was cultured on liquid medium called LB medium \[8\].

**DNA Extraction**

In order to extract DNA, after incubation, the bacterial suspension was centrifuged at 3000rpm for 5min after that the sediment was used for DNA extraction which was performed using promega kit and samples were stored in -20°C. Specific primers were used for PCR and their sequence were as follows:- Amplify the desired sequence, in a volume of 50µl, 2.5 units of Taq polymerase, 50pM of each primer, 200µM of each four deoxynucleotides, 1µl of template DNA, 1.5µM of MgCl₂, 10mM of Tris-Hcl (pH 8.3), and 50mM of KCl were used. PCR was carried out using an Eppendorff’s gradient PCR equipment with the following conditions: 5min of initial denaturation at 95°C, then 40 cycles at 95°C for 30s, 51°C for 1min, 65°C for 8min, and eventually 72°C for 10min for amplification of deficient segments \[9\].

**Preparation of Olive Leaves extract**

The plant extract of the leaves of the olive plant was prepared by mixing 100ml distilled water with 100g of leaves of the olive plant and heated to a temperature of 38°C and left until the work of different
concentrations of the extract after that was made as small tablets of the concentrations of the extract as made to be placed in the colonies of bacteria isolated from fractures of upper and lower jaws and watching of the effect of inhibition of the extract through the diameter of the inhibitory region of the colony [10].

Results

Data from current study showed that five types of inflammatory bacteria were found in upper and lower jaw fractures, they were as the following: *Acinetobacter baumannii* (353 blaOXA-51; 30.05%, *Pseudomonas aeruginosa* (16SrDNA; 36% , *Streptococcus pyogenes* (pSM19035_003 ORF alpha; 30%, *Staphylococcus aureus* (ORF28 similar to putative transposase; 17%. The study found that there were significant differences with different bacterial species with infection rates (Table 1).

**Table (1) Bacterial species isolated from cases of fractures of the lower or upper jaws**

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Number of Cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acinetobacter baumannii</em> (353 blaOXA-51)</td>
<td>51</td>
<td>38.05</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> (16SrDNA sequences)</td>
<td>36</td>
<td>26.8</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em> (pSM19035_003 ORF alpha)</td>
<td>30</td>
<td>22.3</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (ORF28 similar to putative transposase)</td>
<td>17</td>
<td>12.6</td>
</tr>
<tr>
<td>Total</td>
<td>134</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure (1) The package bacteria *Acinetobacter baumannii* (blaOXA-51).
Figure (2) The package bacteria *Pseudomonas aeruginosa* (*16S rDNA* sequences), product size was 984pb.

Figure (3) The package bacteria *Streptococcus pyogenes* (*pSM19035_003 ORF alpha*).

Figure (4): The package bacteria *Staphylococcus aureus* (*ORF28 ~ similar to putative transposase*).
The effect of olive leaf extract was also observed. There was a significant effect among the bacteria treated with olive leaf extract. Concentrations of 10%, 20%, 30%, 40%, 50%, 60% and 70% of olive leaves were used. The concentration of 70% was fatal to all bacteria under study and showed a significant difference (Table 2).

Table (2): The types of bacteria isolated from cases of fractures of the lower or upper jaw and the effect of the plant extract of olive leaves

<table>
<thead>
<tr>
<th>Concentration</th>
<th>10%</th>
<th>20%</th>
<th>30%</th>
<th>40%</th>
<th>50%</th>
<th>60%</th>
<th>70%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumannii (353 blaOXA-51)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (16S rDNA sequences)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Streptococcus pyogenes (pSM19035_003 ORF alpha)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Staphylococcus aureas (ORF28 similar to putative transposase)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

The study showed that there were cases of fractures of the lower jaw or upper jaw of different types were diagnosed using X-ray of the jaws and according to the following images:

![Image (1)](image1)

![Image (2)](image2)
Discussion

A study was conducted to investigate the bacteria that cause inflammation of the lower and upper jaws. In current study, 134 different samples were collected for various fractures. There are different types of fractures; fracture of the angle [11], fracture of the body (molar and premolar region [12] as well as the fractures of the parasymphysis [3]. Types of bacteria causing inflammation were isolated in cases of fractures. The laboratories at the Department of Basic Medical Sciences / Faculty of Dentistry and by using special laboratory techniques for culturing and diagnosis of bacteria represented by the polymerase chain reaction technique and the use of special genes. Five types of inflammatory bacteria were found in the upper and lower jaw fractures, infection with Acinetobacter baumanii appeared with a rate of 51%. This fact may be due to that it could have come from injuries to neighboring countries and this is proven in Iran [14].

The study found that olive leaf extract was effective in eliminating the bacteria that caused inflammation of the lower or upper jaw during the lower or upper jaw fractures. The reason was that the leaves contain phenolic substances that inhibit bacterial growth. The main product extracted from the olive plant is the Olive oil. The Olive oil is one of the bases of the Mediterranean Diet, widely studied for its antioxidant profit from, especially given to the large number of phenolic compounds present in the olive tree. However, the olive plant as well as contain phenolic compounds; the oleuropein, hydroxytyrosol, verbascoside, apigenin-7-glucoside and luteolin-7-glucoside are the most plentiful already identified in olive leaf extracts. The term “Olive fats” refers to a mixture of leaves and bough from both the pruning of olive trees and the harvesting and washing of olives leaves [15].

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of both Environmental and Health and Higher Education and Scientific Research Ministries, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest

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References


