MOLECULAR IDENTIFICATION OF BACTERIAL SPECIES ISOLATED FROM THE NASAL AND OROPHARYNGEAL MUCOSA OF SLAUGHTERHOUSE WORKERS

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ABSTRACT

This study aimed to determine the bacterial species colonizing the nasal and oropharyngeal mucosa of slaughterhouse workers in Central Thi-Qar, Iraq on a microbiological and molecular level. Throat and nasal swab samples were obtained from 20 slaughterhouse workers attendants in the period of time extending from March to May 2021 in Thi-Qar, Iraq. Microbiological identification techniques were utilized to identify the bacterial species isolated. Antibiotic sensitivity was assessed for each of the bacterial isolates. Molecular identification techniques based on PCR. Three bacterial species were isolated from both the nasal cavity and the oropharynx including, Pseudomonas aeruginosa, Staphylococcus aureus and Klebsiella pneumoniae. We found 100% sensitivity of the isolates to ciprofloxacin, cefuroxime and gentamicin. Whereas cefotaxime and azithromycin posted sensitivities of 85.7% and 91.4%, respectively. Low sensitivities (<60% sensitivity) to the antibiotics ampicillin, erythromycin, clarithromycin and norfloxacin were observed. Ninety-seven percent similarity to the microbial bank species was noted when the isolates were compared to it. In conclusion, exposure to meat of animals may be a contributing factor to bacterial colonization of the respiratory tract in slaughterhouse workers.

I. INTRODUCTION

Bacterial waste of meat relies upon the underlying number of microorganism, time/temperature mix of capacity conditions and physicochemical properties of meat (1). Generally, defilement happens on account of deficient clean conditions and taking care of in slaughterhouses (2), besides the connection properties and the biofilm arrangement of microorganisms on surfaces work with cross-pollution (3). Preslaughter conditions like taking care of and lodging including spreadable defilements from skin and defecation, substance of absorption framework, and polluted water are springs of Staphylococcus, Escherichia and Bacillus cereus (2). Various cycles in slaughterhouses like destruction can taint bodies and hardware with gut microscopic organisms (4). The most usually recognized fecal coliforms in slaughterhouses are: Enterobacter, Citrobacter and Klebsiella (2; 5; 6). Anti-infection safe enterococci were reliably disconnected from steers, poultry and pig cadavers, or from new meat (7). Pseudomonas are the major causative deterioration microorganisms in meat, basically because of their metabolic adaptability and capacity to deliver extracellular proteases and lipases cause oxidation, shading change, off-flavor, disgusting or soft structure and creature tissues debasement (8; 1). Schlegelova et al. (2) announced that tainting of meat by safe strains of S. aureus and E. coli during butchering measure drastically have been expanded showing, optional defilement from the climate of slaughterhouses (2). Culture subordinate techniques can inadequately decide biological specialties and cooperative connections, additionally mutilating the local area structure of the cultivable part during refined because of selectivity (9). In this examination, we explored microbial variety on various surfaces and gear in slaughterhouses and laborers.

II. MATERIALS AND METHODS

Sample collection and isolation and testing for antibiotic resistance

Participants were recruited from slaughterhouse in Thi-Qar, Iraq, during the period of time between March and May 2021. All the participants were informed about the aim and objectives of the study and approval forms were obtained. The swab samples were then inoculated onto special agar specific for the diagnosis. This method
detects alpha hemolytic abilities and removed the need to inoculate stains onto blood agar plates and interpret their pattern of hemolysis thus leaving room for error in interpretation. PCR molecular analysis and the phylogeny representation were performed on 10 randomly selected samples from the colonies isolated.

**Blood sample collection and analysis**

Blood samples where drawn into EDTA tubes (lavender tops) under clean conditions. Samples were stored in a potable cool container and processed in the lab within 1 h of collection.

**DNA extraction and sequence analyses**

DNA was extracted from isolates using the CTAB (N-capyl-N,N,N trimethyl ammonium bromide) method described by Murray (The Human Microbiome Jumpstart Reference Strains, 2010) respectively: *Pseudomonas aeruginosa* f (ATCGGCACTCTTATGGCAGC) R(GCCCTTGGCTGGTCATTACT)(502bp) Klebsella pneumonia F (CGCGTTGGCTATCGTTTCAG) R (AATGCCCAACCTAGTTCCG) (527 bp) and *Staphylococcus aureus* F (CAAAATGGTCGTGCAGCACA) R (AAGCGGTTATGAGTGCCACA) (522 bp).

PCR products were purified using the QIA quick PCR purification kit (QIAGEN, GmbH, Germany), and sequenced in both directions using the respective PCR primers. For this purpose, All PCRs and sequencing reactions were performed on a GeneAmp PCR System 9700(Applied Biosystems).

**III. RESULTS**

Twenty samples were collected from slaughterhouse workers, where the results of the current study showed the diagnosis of three types of bacteria, which are as shown in Table. 1, Fig (1,2,3), where the rate of infection with *Pseudomonas aeruginosa* was 10 infected from 20 people, as well as in the *Staphylococcus aureus* bacteria, the same disease, whereas in the *Klebsiella pneumoniae* bacteria the number and percentage were The infection was less, and it was 7 people out of 20 samples.

![Figure 1](image1.png)

Figure (1): Agarose gel electrophoresis image that showed the PCR product analysis of 18S ribosomal RNA gene in *Pseudomonas aeruginosa* isolates. Where Marker ladder (1500-100bp), 16rRNA gene in *Pseudomonas aeruginosa* isolate at 502bp PCR product size.
Figure (2): Agarose gel electrophoresis image that showed the PCR product analysis of 18S ribosomal RNA gene in *Klebsiella pneumoniae* isolates. Where Marker ladder (1500-100bp), 16rRNA gene in *Klebsiella pneumoniae* isolate at 527bp PCR product size.

Figure (3): Agarose gel electrophoresis image that showed the PCR product analysis of 18S ribosomal RNA gene in *Staphylococcus aureus* isolates. Where Marker ladder (1500-100bp), 16rRNA gene in *Staphylococcus aureus* isolate at 527bp PCR product size.

Table 1 Bacteria isolated from 58 samples from the nasal cavity and oropharynx in slaughterhouse workers.

<table>
<thead>
<tr>
<th>No.</th>
<th>Bacterial isolates</th>
<th>Total</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>20</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>20</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td><em>Staphylococcus aureus</em></td>
<td>20</td>
<td>10</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 2 details the results of the bacterial sensitivity and resistance testing of the bacterial isolates to commonly used antibiotics. A 100% sensitivity of the isolates to ciprofloxacin, cefuroxime, gentamicin and imipenem was found when the samples were tested against these antibiotics, whereas with cepotaxime, azithromycin and doxycycline, sensitivities of 94.3%, 91.4% and 90.5% respectively were recorded. Low sensitivities (less than 60% sensitivity) to the antibiotics ampicillin, erythromycin, clarithromycin, norfloxacin and cefaclor were observed. These antibiotics were tested only in less than 20% of the bacterial isolates, except for norfloxacin, which was tested in 66.1% of the isolates. Ten bacterial isolates were randomly selected to undergo the process of gene sequencing and comparison.

Table 2 Bacterial sensitivity and resistance to antibiotics of all samples (n= 58).

<table>
<thead>
<tr>
<th>No.</th>
<th>Antibiotic (antibiotic identification card Vitek)</th>
<th>No. of samples tested</th>
<th>Percentage of sample tested (%)</th>
<th>Sensitivity (%)</th>
<th>Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ciprofloxacin</td>
<td>47.0</td>
<td>81.0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>Cefuroxime</td>
<td>39.0</td>
<td>67.2</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>Gentamicin</td>
<td>15.0</td>
<td>25.9</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>Imipenem</td>
<td>3.0</td>
<td>5.2</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>Cefotaxime</td>
<td>42.0</td>
<td>72.4</td>
<td>85.7</td>
<td>14.3</td>
</tr>
<tr>
<td>6</td>
<td>Azithromycin</td>
<td>35.0</td>
<td>60.3</td>
<td>91.4</td>
<td>8.6</td>
</tr>
<tr>
<td>7</td>
<td>Doxycycline</td>
<td>42.0</td>
<td>72.4</td>
<td>90.5</td>
<td>9.5</td>
</tr>
<tr>
<td>8</td>
<td>Ampicillin</td>
<td>13.0</td>
<td>22.4</td>
<td>53.8</td>
<td>46.2</td>
</tr>
<tr>
<td>9</td>
<td>Erythromycin</td>
<td>12.0</td>
<td>20.7</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>10</td>
<td>Clarithromycin</td>
<td>5.0</td>
<td>8.6</td>
<td>40.0</td>
<td>60.0</td>
</tr>
<tr>
<td>11</td>
<td>Norfloxacin</td>
<td>41.0</td>
<td>70.7</td>
<td>59.7</td>
<td>6.5</td>
</tr>
<tr>
<td>12</td>
<td>Cefaclor</td>
<td>4.0</td>
<td>6.9</td>
<td>50.0</td>
<td>50.0</td>
</tr>
</tbody>
</table>
As of late, the study of disease transmission of microorganisms and its recently arisen safe strains has acquired consideration in both veterinary and human medication, especially due to their zoonotic potential. Albeit the development and spread of safe Staphylococcus strain and Pseudomonas aeruginosa and Klebsiella pneumoniae has been recently revealed from obviously sound pets [10] and pigs [11], there are no authoritative information on its commonness in clearly solid camels or their job as transporters. In this examination, secluded from half (10/20) of the 20 slaughterhouse laborers, who were working transcendentally at the researched abattoirs (Table 1). Fundamentally the same as S. aureus confinement rates (11.7%) were accounted for in cadaver swabs from abattoirs in Addis Ababa, Ethiopia [12]. Nonetheless, the general S. aureus and predominance in this examination was lower than that announced from nasal examples from camels in Nigeria (20.7%) and higher than that detailed in human contacts (11.5%) in a similar report [13].

Over the previous decade, the issue of antimicrobial opposition in the African mainland has acquired unique interest. In any case, little is thought about the genuine degree of the issue since observation for antimicrobial obstruction is completed in a couple of nations [14]. In this investigation, the entirety of the got microscopic organisms disconnects showed various examples of multi-protection from the tried antimicrobials. The most widely recognized obstruction designs separates human disengages (Table 2). The development of such safe strains assumes a significant part in remedial disappointment in both human and creature diseases. The uncontrolled utilization of anti-toxins in human and creatures, along with poor indicative procedures and unseemly endorsing by unfit doctors, intensifies the issue [15] and comprises an extraordinary test for the anticipation and control of this microorganism. A similar obstruction design was recently noted in MRSA detaches from an emergency unit Hyderabad, southern India, by utilizing the circle dissemination strategy [14]. Also, as of late in India, Bacteria was recognized in 16.7% of MRSA segregates acquired from bison nasal and skin tests by utilizing the plate dissemination strategy [16]. Taking into account this anti-microbial obstruction, VAN is presently a lastchoice anti-toxin for the treatment of MRSA, and its utilization in human and creatures is restricted [16, 17]. As of late, because of the presentation of other elective mixtures, VAN is at this point not an anti-toxin after all other options have run out; in any case, it is the most often utilized anti-microbial in instances of staphylococcal contaminations [18]. Despite the fact that the Bacteria strains were believed to be uncommon as of not long ago [19], Moreover, animals related MRSA (LAMRSA) has been recently identified in the kin of ranchers who were in touch with animals [20], recommending an expected danger for zoonotic transmission to contacts [21]. Moreover, other past examinations showed the procurement of LA-MRSA from taking care of meat in Hong Kong [22, 23]. The vast majority of these kinds of defilement occasions are of more prominent concern in Asia and Africa than in Europe, the USA, and Canada [24]. Microscopic organisms was secluded from contaminated or colonized people in Turkey and Asiatic nations [25,26–27,82]. Albeit the front nares are generally the essential site to evaluate for S. aureus, 90% of human nasal transporters additionally present colonization on their hands [29]. The unmistakable limit of this investigation was the absence of nasal swabs from the nasal swabs from the laborers; the last would have been significant concerning microscopic organisms colonization and the danger for additional spread among human. Another constraint was the absence of clonal portrayal of the Bacteria strains segregated from human and creatures. A large portion of these microorganisms, if resistant protections were lost or debilitated, might actually be the reason for respiratory and non-respiratory framework diseases. The anti-toxins to which the microorganisms showed total affectability included extremely wide range anti-toxins and is not the slightest bit characteristic that contaminations with these organic entities when and in the event that they happen will be not difficult to treat with first line specialists.

V. CONCLUSION

The present study reported the presence of bacteria in human in contact with meat. Our research is the first in Iraq to report bacteria in slaughterhouse workers, and we urge further comprehensive molecular epidemiological surveillance studies on the extent and potential zoonotic transmission of bacteria in livestock animals. Urgent interventions to control the transmission of these antibiotic-resistant organisms in abattoirs are needed.

REFERENCES


