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Isolation of proteus spp. bacterial pathogens from raw minced meat in Alkarkh area, Baghdad prevalence

Mohannad Sami Al-Kubaisi
Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad, Iraq

Ahmed Husam Al-Deri
Department of Veterinary Public Health, College of Veterinary Medicine, University of Baghdad, Iraq
Corresponding author email: mohanadalkopisi@gmail.com

**Abstract**---The aim of the study was to isolate *proteus* bacteria species from raw minced meat obtained from some butcheries in Alkarkh area. A total of 60 raw minced meat samples were collected from meat shops in the Alkarkh area-Baghdad Province/Iraq. The samples were analyzed for the presence of *proteus* spp. bacteria using MacConkey, Blood, and Selective agar. A total of isolates that satisfied the preliminary biochemical tests were further confirmed by using the API 20E assay, Vitek2 Diagnostic Method, and a final confirmation test by polymerase chain reaction (PCR). The proportion of *proteus* spp. bacteria was 8.3% obtained from all the bacteria isolated in minced meat samples. Although most of the species identified are pathogenic to humans, some strains are known to foodborne outbreaks cause even in countries with proper public health facilities. It is recommended that effective food safety education and training of personnel that handle food at retail points will help to reduce the effect of these pathogens on humans.

**Keywords**---isolation, *proteus* spp., bacterial pathogens, raw minced meat.

**Introduction**

Meat is a nutritious source of protein for humans and contains numerous compounds such as phosphate, vitamins, fat, water, protein, and iron. The majority of the meat has a lot of water, which encourages germs to multiply (Rao VA *et al*., 2009). Meat can be contaminated by a variety of factors, including the
environment, human handling, manipulation, and/or the animal itself (Marritto and Gravani, 2006). The Enterobacteriaceae bacterium family is the most difficult to eradicate from meat and meat products around the world. In all cases of food poisoning linked to meat products, E. coli, Salmonella, Proteus, and Klebsiella are the most common bacteria (Drzewiecka, 2016 and Nahar et al., 2014). Many food poisoning outbreaks have been linked to Proteus group organisms, and with the increased incidence of Proteus spp. Related food borne diseases, there is an urgent need for control and/or prophylactic for food poisoning outbreaks linked to meat products. To ensure food safety and protect public health from microbial contamination of food, it is critical to investigate such agents in foods and eliminate them (Cooper et al., 2005).

The family Enterobacteriaceae includes the genus Proteus. P. mirabilis, P. vulgaris, P. penneri, and P. myxofaciens are the four known species of Proteus. P. vulgaris is the most commonly isolated species from clinical illnesses and opportunistic infections (Zappa et al., 2017). Proteus species are different from most of other genera by their ability to swarm through a blood agar surface (Mohammed et al., 2016). These bacteria are Gram negative bacillus, non-capsulated, non-spore forming, Lactose non-fermenting, catalase positive, oxidase negative, facultative anaerobic, hydrolyze urea quickly, chemo-organotrophic having together a respiratory and a fermentative form of metabolism (Ahmed, 2015).

The Proteus bacteria found as normal flora in the intestinal tract being as saprophytes, whereas a number of them may present as parasitic status and might be an opportunistic pathogens, which producing numerous types of infections when they leave their natural inhabitation (Drzewiecka, 2016). Proteus species, like several other members of the Enterobacteriaceae family, are short (1.5- to 2-μm) straight rods that exhibit dimorphism as "swimming" and "swarming" forms (Armbruster and Mobley, 2012)

The best temperature for the growing of Proteus spp. is 37 °C with a pH 7.4 many culture media have been developed for diagnosis of Enterobacteriaceae, which in turn can discriminate between genera in this family, including Proteus spp. (Chen et al., 2015). The bacteria seemed as distinct pale colonies on MacConky agar (Mohammed et al., 2016). Non-ferment lactose, moderate in size and entire edges colony as well as the odor of bacterial growth which is similar to odor of fish decaying, which is the formula primary diagnostic for this bacteria and performed swarming movement on the blood agar. Swarming behavior makes it hard to isolate as the distinct colony, this phenomena can restrained by increased concentration of agar (2.5-3.0%) or P- nitrophenylglycerol considerably restrained swarming but its influence on growth amount was not important (Kadhim et al., 2019).

**Materials and Methods**

**Collection of samples**

Sixty samples were collected from different areas in Baghdad, capital of Alkarkh (Al-ameria, Al-Ghzalia, Al-shoala, Hey aljehad, Hey alhussian, Hey alamel, Albyaa, Hey-Aladel, Alhuria, Al-Alawe, Hey-alkadraa, and Al-Manssor city). The samples
were collected randomly from butcher shops in these areas and transported to the veterinary meat hygiene laboratory from November 2021 to February 2022. The samples were collected in sterile Zip-lock polyethylene bags in the morning hours and transported to the laboratory in an ice box for analysis and processing immediately.

**Isolation and Identification of bacteria**

**Sample collection**

All the 60 minced meat samples collected were inoculated in nutrient broth (1:10) dilution (food standard formula) as one part sample (25 grams) to nine parts (225 ml) of broth. Then it was homogenized with a stomacher for 3–5 minutes, and then incubated at 37°C for approximately 18–24 hours (Al-Rhada, A. M., 2020).

**Culture of minced meat samples**

The method was applied to isolate *Proteus* spp. Collected sample for isolation and characterization of bacteria pathogens from minced meat and carried to the laboratory of Department of public health in Veterinary Medicine College /University of Baghdad. the broth was cultured directly by streaking on selective and differential media, including as MacConkey Agar, blood base agar, and selective media incubated aerobically overnight at 37°C. Visual analysis of the shape of the bacterial colonies was used to evaluate primary cultures. subcultured once more, and stained with Gram stain. (Sadeq et al., 2018).

1- Microscopic Examination(Gram Stain):
   Gram stain was used to stain all bacterial isolates, according to Thomson (2016).
2- Biochemical Tests: Catalase Test, Oxidase Test, Urease Activity Test, Simmon citrate agar, Triple Sugar Iron Agar(TSI), Motility Test, Gelatin liquefaction Medium and Phenyl alanine Deaminase Agar.
3- API 20 E Diagnostic Systems: This system is based on 20 biochemical assays specific to Enterobacteriaceae and was used in accordance with the manufacturer's instructions (Shi et al., 2017).
4- Vitek2 Diagnostic Method: These devices include 64 biochemical tests which are used in the diagnostic of bacteria to reach the 98% degree of accuracy (Hogan et al., 2019).
5- Molecular Detection of Isolated *Proteus* spp.by PCR: Molecular identification of the genus proteus was performed by partial amplification of the gene *rpoB* using specific primers designed in this study by Primer3Plus bioinformatics tool. The primers were manufactured by BioNEER (Korea). They were checked by using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI). The primer sequences are mentioned in Table 1. Conventional PCR was used to confirm the identity of some isolates obtained from food.
Table (1): Primer’s sequence of rpoB gene

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Tm (°C)</th>
<th>GC (%)</th>
<th>Product size(bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward</td>
<td>5’-GAAACGCCTACCGTGTGT-3’</td>
<td>60°</td>
<td>50.0</td>
<td>240 bp</td>
</tr>
<tr>
<td>Reverse</td>
<td>5’-CAGTGAAGCACCGACAGAAA-3’</td>
<td>60°</td>
<td>50.0</td>
<td></td>
</tr>
</tbody>
</table>

**Results**

1- Culture characteristics
2- MacConkey agar, giving non-fermented lactose, the result was a medium converted to yellow and giving a moderately sized, convex, round with smooth edges colony as well as the odor of bacterial growth, which is similar to the odor of decaying fish, as in figure 1.

![Figure 1: Growth of Proteus spp. on MacConkey agar](image)

blood agar and the growing colony with significant pattern swarming motility was a key feature that distinguished it from other Enterobacteriaceae strains on blood agar. As shown in the Figure 2
3- Biochemical Identification

The results shown in Table 2 that these isolates were positive for catalase, urease, phenylalanine, gelatin, and motility, but negative for oxidase. and vibration in Simmons Citrate according to proteus strains. Bacteria showed their ability to produce H2S and gas when they were cultured on Triple Sugar Iron (TSI) agar while the slant turned red and the bottom was yellow.
Table 2: Identification results for suspected *Proteus* spp. isolates. (+) positive result; (-) negative result; (K/A)Alkaline/Acidic

<table>
<thead>
<tr>
<th>No.</th>
<th>Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gram Staining</td>
<td>-ve</td>
</tr>
<tr>
<td>2</td>
<td>TSI</td>
<td>K/A H2S +Gas -</td>
</tr>
<tr>
<td>3</td>
<td>Catalase</td>
<td>+ve</td>
</tr>
<tr>
<td>4</td>
<td>Oxidase</td>
<td>-ve</td>
</tr>
<tr>
<td>5</td>
<td>Urease</td>
<td>+ve</td>
</tr>
<tr>
<td>6</td>
<td>Phenylalanine deaminase</td>
<td>+ve</td>
</tr>
<tr>
<td>7</td>
<td>Motility</td>
<td>+ve</td>
</tr>
<tr>
<td>8</td>
<td>Gelatinase</td>
<td>+ve</td>
</tr>
<tr>
<td>9</td>
<td>Simmons Citrate</td>
<td>+ve or –ve</td>
</tr>
</tbody>
</table>

Figure 4 *proteus* spp. gives Urease positive

Figure 5 *proteus* spp. give Simmons Citrate positive

Figure 6 *proteus* spp. Gives TSI Test positive

Figure 7 *proteus* spp. Gives Phenylalanine positive

Figure 8 *proteus* spp. give Motility Test positive

Figure 9 *proteus* spp. give gelatinise test positive
4- all positive sample give positive result in confirm test (API20E Systems & Vitek2 Diagnostic)
5- The polymerase chain reaction (PCR) technique successfully amplified the \textit{rpoB} gene of \textit{Proteus} spp. isolated from five samples. Figure 10 shows bands of approximatel 240bp, as expected, indicating presence of this bacterium in minced meat.

![Figure 10: Agarose gel electrophoresis images, A and B, show bands of the PCR product of the partially amplified \textit{rpoB} gene of \textit{Proteus} spp. M: DNA ladder (100-1500 bp), lanes 1,2,3 and 3,4 are 240 bp of the target gene.](image)

From all the sixty (60) tested samples, only five (8.33\%) were shown to be positive for \textit{proteus} spp. by presumptive isolation and confirmatory testing. The result was agreed with Noha \textit{et al.} (2014) who identified \textit{proteus} spp. that were isolated from minced meat.

**Discussion**

The presence of high \textit{proteus} spp. counts in minced meat indicates poor sanitary conditions inside the butcher's shops especially for mincing machines which were used for meat mincing without periodical washing or cleaning and also workers hands which carry heavy contamination and contaminate meat by bad handling (Noha \textit{et al.},2014). It is clear from the previous results that the \textit{Enterobacteriaceae} counts seem to be high and this draws our attention to the contamination from enteritis sources so it can be used as proof for enteric contamination (Mercuri and Cox., 1979).

\textit{Proteus} spp. are widely distributed in the environment with reservoirs. They are opportunistic pathogen, nevertheless, under favorable conditions they can cause urinary tract infection, which may lead to sever complication such as pyelonephritis or stone formation (JebaMercy \textit{et al.}, 2019). \textit{Protues} spp. is one of the intestine's pathogens which are transmitted to human through food specially
contaminated meats. The infected meat has no apparent symptoms of putrefaction and eating of such meat in raw and/or undercooked forms can lead to acute gastroenteritis, dysentery, mesenteric lymphadenitis and even septicemia (Razavilar, 2003; Gupta et al., 2015; Laura et al., 2019). In Iraq, few published data about meat contamination with Proteus spp. In spite of it is isolated from patients suffering from enteritis (Kanan and Abdulla, 2009). This study recognized the incidence of Proteus spp. In minced meat through performing cultural, biochemical, complementary and molecular tests.

References


