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

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***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 Enterobacteriaecae 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 37C^{o} 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^{o}. 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 *rpo*B gene

Primer	Sequence	Tm (C°)	GC (%)	Product size(bp)
Forward	5'-GAAACGCCTTACCGTGTTGT-3'	60° C	50.0 %	240 bp
Reverse	5'-CAGTGAAGCACCGACAGAAA-3'	60° C	50.0 %	

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.

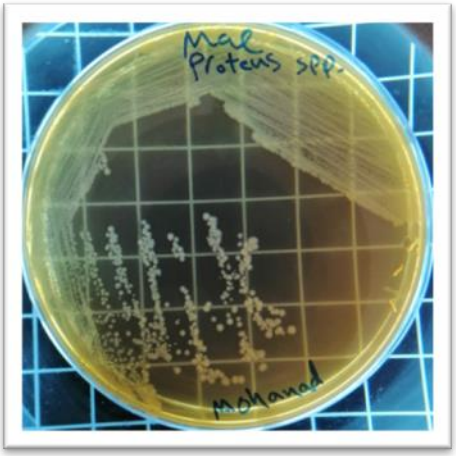

Figure 1: Growth of Proteus spp. on MacConkey agar

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

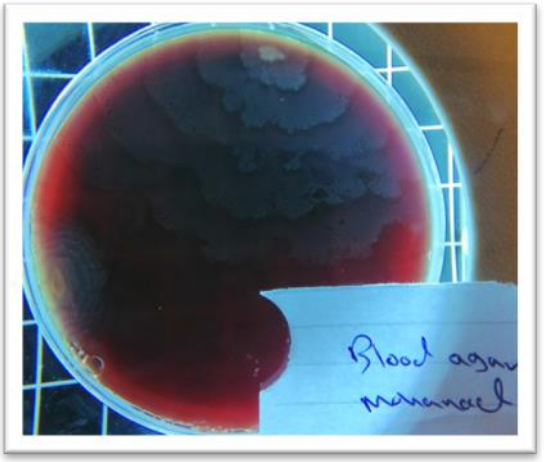

Figure 2: Growth of *Proteus* spp. on blood agar (swarming motility feature)
selective media Colonies of Proteus spp. were moderate, large, thick, greyish-white, circular disks that were dome-shaped and smooth. As shown in Figure 3

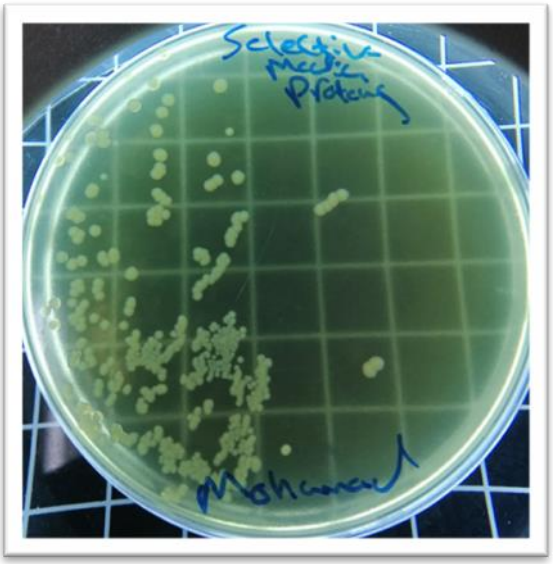

Figure 3: Growth of *Proteus* spp. on selective media

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

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

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 Motality 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 *rpo*B gene of *Proteus* spp. isolated from five samples. Figure 10 shows bands of approximatel 240bp, as expected, indicating presence of this bacterium in minced meat.

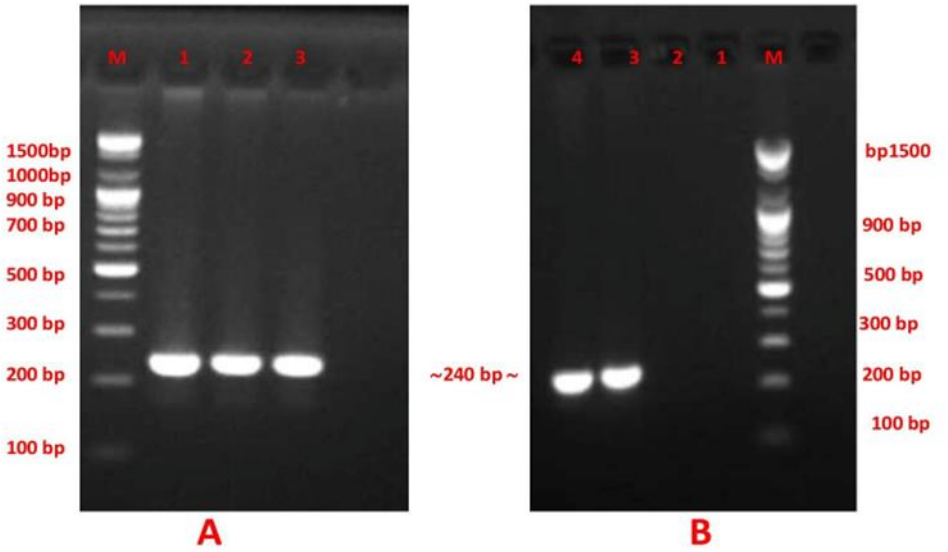

Figure 10: Agarose gel electrophoresis images, A and B, show bands of the PCR product of the partially amplified *rpoB* gene of *Proteus* spp. M: DNA ladder (100-1500 bp), lanes 1,2,3 and 3,4 are 240 bp of the target gene.

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

Discussion

The presence of high *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 et al.,2014). It is clear from the previous results that the *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).

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 et al., 2019). *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 *protues* spp. In spite of it is isolated from patients suffering from enteritis (Kanan and Abdulla, 2009). This study recognized the incidence of *protues* spp. In minced meat through performing cultureing , biochemical, complementary and molecular tests.

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