Isolation &Identification of *Proteus* spp. bacteria isolated from Locally processed raw Beef in Baghdad City

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Abstract

This study was done to demonstrate the bacterial contamination of many type of raw Beef meat products that processed locally in Iraq by *Proteus*. spp. bacteria , during January to June from 2022 one hundred fifty Beef products samples ((burger, meatball, pasterma) were randomly purchased from different local markets in Baghdad City ,These samples were sent to the Lab. of Microbiology for bacterial identification which performed by using different selective media like MacConkey Agar, blood agar, XLD agar, more identification was done by using Gram stain technique and biochemical tests , (11) isolates of *Proteus* spp. as (7.33%) was diagnosed and confirmed as *Proteus mirabilis* using VITEK 2 compact technique & Finally the Nucleotide Sequence of 16S rRNA Gene of isolated bacterial spp. was identified by PCR technique.

Key word: *Proteus mirabilis*, Nucleotide Sequence ,16S rRNA.

Introduction

Animal tissues is the main source of meat ,in general all internal part of animal tissues could be considered as sterile before it introduced to slaughtering [1,2] when beef meat exposure to slicing and cutting that will lead to high possibility for contamination in comparison to meat as a carcass [3,4]. Any pathogenic microorganism even that found in a small number in meat and other edible part may lead to high contamination during processing and the surfaces of meat will exposed to more contaminant of pathogens [5,6].

Many complex condition control the shelf life of meat products which mean the time from storage to appearance of spoilage signs ,the interaction of these chemical , biological and physical factors will lead to unacceptability of meat products to the human as a food [7,8]. The Physiological statue of livestock may lead to growth of various species of microorganisms specially during farming , slaughtering ,processing ,harvesting , transport, preservation and storage. [9,10].

Properly cooked for meat and meat products should be performed to avoid food borne diseases specially these caused by enteric bacteria in addition to that some of these microorganisms are psychrophiles and can produce toxin which lead to the food spoilage if meat products were badly refrigerated and during transport [11,12,13].

Strict Hygienic strategies during processing and handling meat and meat product are a major factor that obviously influenced the microbial quality of beef meat , environmental factors in butcher shops and industrial facilities can be a significant source of microbial contamination. [14,15,16]. One of the major causes of food-borne diseases is tainted raw meat. [17,18].

Members of family *Enterobacteriaceae* like *E. coli*, *Salmonella* spp., *Klebsiella* spp. and *Proteus* spp.could be the main cause for beef and chicken meat contamination with fecal material , this may be considered as a serious issue in the field of public human health [19,20,21]. Genus *Proteus* bacteria ecologically are wide distribution. They are present in contaminated soil, water, and manure, where they are crucial to the breakdown of organic material of an animal origin [22, 23,24].

Members of genus *Proteus* consists of five named species (*P. mirabilis, P. penneri, P. vulgaris, P.myxofaciens, and P. hauseri*), *Proteus mirabilis* and *Proteus vulgaris* bacteria were considered as an opportunistic agents for wound infection and respiratory disease in addition to the urinary tract infection [25] , In addition to the wide distribution of bacteria in the environment *Proteus* bacteria also considered as an important flora of both human and animal intestinal tract which allow them to be a source of an opportunistic infection, recently this bacteria may be related to the multidrug resistance pathogens [26]. It can also cause gastrointestinal inflammation due to the ingestion of contaminated meat and other contaminated, food ,Bacterial Food born illness was induce due to consumption of food that was contaminated with bacteria or its toxins or both [27] Many studies refer the serious problem of contamination of food from animal origin with *Proteus* bacteria specially meat due to the dissemination of virulent genes of this bacteria so workers who involved on the sector of meat industry like farmers , butchers should be aware about preventive measures during collection , handling and transport meat products [28] .One of the big obstacles in the food safety industry is the food born disease of pathogenic microorganisms which may cause a big burden on public health each year [29,30].

Materials and Methods

Isolation &Identification Of bacteria

Collection of Samples

One hundred fifty (150) of raw beef products samples Locally (meatball,burger ,pasterma), were collected from different markets in many quarters on Baghdad city and sent to microbiology Lab. on a sterile condition for primary isolation of *Proteus* bacteria *spp*.

Bacterial Isolation

From each Beef sample (25) grams was taken randomly and homogenized in a sterile conditions inside the cabinet , after that (3) g from each sample should be inoculated in (5) ml of nutrient broth in sterile tubes , all tubes were incubated all over night at 37°C hours. then a loopfull was taken from each tube and cultured on a different selective media like Xylose Lysine Deoxycholate agar (XLD), MacConkey Agar and Blood

agar, all plates were incubated for 18-24 hours at 37°C, the bacterial colonies shapes ,color, size, texture and other morphological characteristics should be examined visually , eventually a selective colony was stained by Gram stain for microscopic examination.

Biochemical Test for Bacterial Identification

Many biochemical test should be done by select a loopfull colonies from already cultured media like MacConkey agar , using a straight metal loop a loopfull colony inoculated, on a slant urease prepared media, Triple sugar iron and Simons citrate agar other test also done like Indole test , Motility test oxidase and catalase test [31].

Confirmation Test by VITEK 2 Compact System

Eleven (11) bacterial isolates that identified by cultural diagnosis and biochemical test as *Proteus* spp. bacteria was tested by Gram negative cards of the VITEK 2 Compact device, diagnosis depend on sixty four test found in the device with accuracy rate 98 %,two colonies of already cultured media were suspended on 3ml of normal saline, the turbidity was adjusted to 0.5 McFarland standard ,all tubes and diagnostic cards should be put on the racks and entered to the device in which the diagnostic results could be read after 18 hours [32].

Molecular Detection by PCR

The DNA bacterial isolates was detected from overnight growth , the gene sequence was confirmed through rRNA database (NCBI), the procedure of bacterial deoxyribonucleic acid extraction, the amplification ,the sequencing and assembly. 16S rRNA using 27F and 1492R primers; and yielding 0f 1,3000 pb or more data of sequencing was used for Bacterial PCR [33]. The DNA that produce on PCR were analyzed by Sanger sequencing which use AB13730XLfor , Macrogen Corporation – Korea automated the sequencer deoxyribonucleic acid ,The primers sequencing which designed by Macrogen Corporation– Korea was shown on (Table1) are :

Table (1): The primers used on study and their sequence

Primer Name	Seq.	Annealing Temp (°C)	Product size (bp)
27F	5`-AGAGTTTGATCCTGGCTCAG-3`	60	1500
1492R	5`-TACGGTTACCTTACGACTT-3`		

Results and Discussion

Cultural and Microscopic Characteristics

The morphological characteristics of the isolates were identified in Blood media by forming rings of growth cycles due to their swarming phenomenon ,colonies growth were pale strew color on MacConkey agar and yellowish colonies with hydrogen sulfide on XLD agar because bacteria not ferment lactose , microscopic examination reveals gram negative , rod , straight bacteria when gram stain was used ,The cultural and microscopic characteristics of bacteria are shown in (Figure 1).

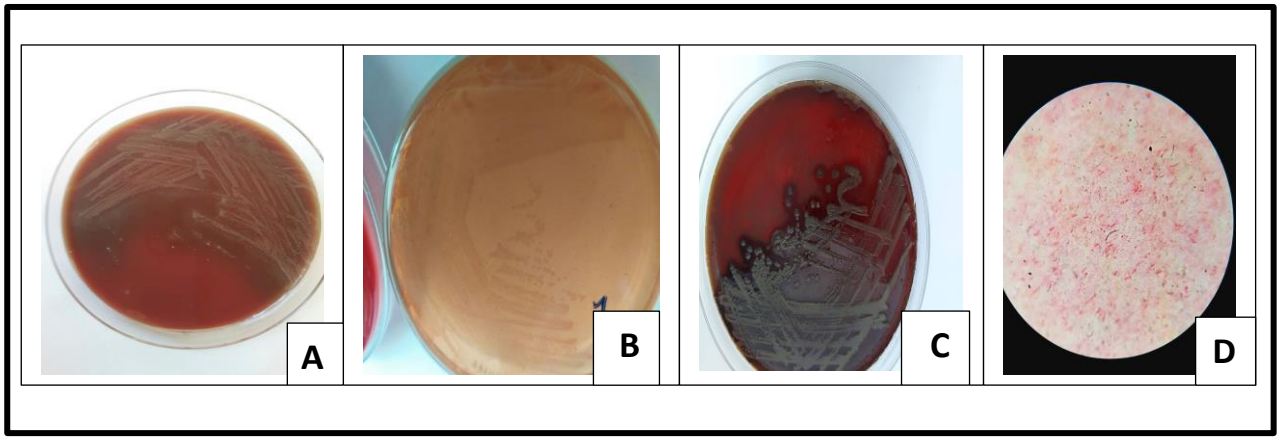

Figure (1): The *Proteus* bacteria colonies morphology on : A) Blood agar, B) MacConkey agar C) XLD agar , D) Gram stain under microscope

Table (2) : The biochemical test of Proteus spp. bacteria

NO.	Name of Test	The change on the Media
1	Urease	(+) bacteria have urease which utilize urea to NH3.
2	TSI	Y/Y bacteria utilize glucose to acid on slant and button with H_2S production
3	Citrate Utilization	(+) bacteria utilize citrate as a source of carbon
4	Indole test	(-) bacteria do not have tryptophanase enzyme,
5	Motility test	(+) *Proteus* spp. are motile bacteria.
6	Catalase test	(+) bacteria have catalase enzyme
7	Oxidase test	(-) bacteria do not have cytochrome oxidase enzyme

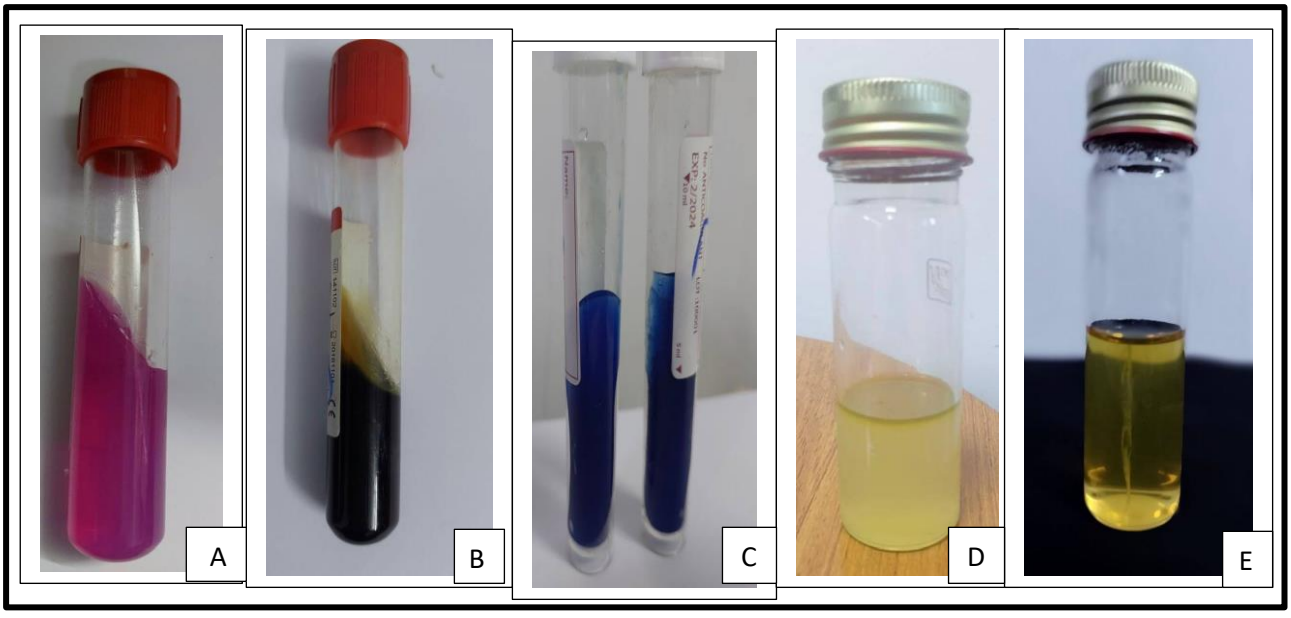

Figure (2) : The Biochemical test of Proteus spp.: A) Urease test B) Tri-sugar- iron test, C) Citrate utilization test , D) Indole test. E) Motility test.

Diagnosis by VITEK 2 Compact System and PCR Technique

VITEK 2 compact device reveals that all the (11) *Proteus* spp. isolates which was diagnosed by cultural and biochemical test are *Proteus mirabilis* which represent as (7.33 %) from the all samples of meat products ,That is resemble to what [34] found on their study and that was confirmed by PCR technique amplification the bacterial genome and using a specific primer. as shown in (Figure 3) .

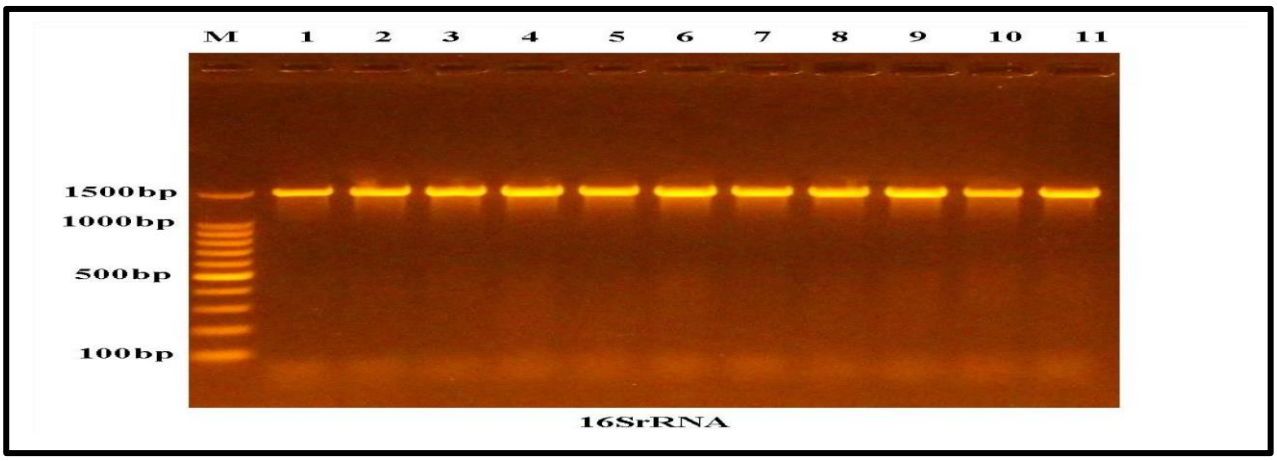

Figure (3): The DNA of bacteria after amplification

From total (150) collecting samples (11) isolates in a ratio (7.33%) were diagnosed as *Proteus mirabilis* bacteria during the period of the study distributed on different months and various quarters on Baghdad city as shown on (Table 3) and (Table 4) which was close to the percentage of *Proteus mirabilis* isolation from beef meat on studies done by [35,36].

Table (3): The months of samples collection shows no significant differences with the isolation percentage.

The month	Total Samples	# of *Proteus mirabilis* Isolates	The % of the isolates	Yates' *p*-value	Yates' Chi-square value
January	20	1	0.66%	0.20	0.99NS
February	20	2	1.33%		
March	30	3	2%		
April	25	1	0.66%		
May	45	4	2.66%		
June	10	0	0 %		
Total	150	11	7.33%		

Table (4): Quarters of samples collection on Baghdad City shows no significant differences with the isolation percentage

Name of Quarter	# of Samples	+ Result to *P. mirabilis*	% of *P. mirabilis*	Yates' *p*-value	Yates' Chi-squa Value
Hey Alaamel	35	5	3.33%	2.70	0.91NS
Hey Al- Hussain	25	3	2%		
Albayaa	10	0	0%		
ALKhadamiyah	20	1	0.67%		
AL Yarmooq	15	0	0%		
Hey Al Jameah	10	0	0%		
Alameria	15	0	0%		
AL Maamoon	20	2	1.33 %		
The Total	150	11	7.33		

Many studies mentioned that most foodborne diseases were caused by pathogenic microorganisms from animal origin [37]. Most food establishment that has proven to be contaminated with pathogenic microorganisms is an indication for poor meet handling during processing [38] Beef meet contamination with animal fecal material may be the main source of *Enterobacteriacea* [39,40]. During slaughtering process may bacterial genus belongs to *Enterobacteriacea* could cause sever contamination to the carcasses specially during cutting ,mincing, packaging [41,42,43] that will cause a serious health problem and may be related to many food poisoning outbreaks which related to beef meet consumption. [44]. Many researches pointed that *Proteus mirabilis* bacteria considered one of major cause of urinary tract opportunistic disease and may give rise to septicemia through wounds. Beside that this bacteria could be a reason for food poisoning when consumed in contaminated meet and other food [45]. In last years only a few cases of food caused by *Proteus* bacteria have been reported [46,47] However a study by [48] which isolated a new strains of *Proteus mirabilis* from patients suffering from sepsis, food poisoning ,peritonitis and meningitis ,this study has been reported an immerged strains of bacteria with virulent pathogenesis which can cause a a big risk to the human health and may be a major reasons for serious diseases ,Also in another study [49] who mentioned that more and more cases of food poisoning related to *Proteus mirabilis* bacteria have been reported on recent years , The unfirmly meat hygiene lead to high and remarkable percent of *Proteus* spp. that isolated from different samples of raw beef meat products which reach to 58 % in many cases reported on a study in Egypt. [50].a study by [51] concluded that *Proteus mirabilis* was the most frequent between gram negative bacteria that isolated from beef carcasses which in turn lead to reduce the meat quality and could play an important role in the public health concern .

Although there are few studies refer to the role of *P roteus* spp. bacteria in food contamination , but in this work (11) as (7.33 %) isolates of *Proteus mirabilis* has been

isolated from (150) beef samples processed locally that was identified and confirmed by microbial laboratory diagnosis, VITEK 2 system and finally PCR technique , which in turn reveals that *Proteus mirabilis* bacteria may be considered as one of an important bacteria that cause meat contamination to the beef meat products.

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