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Microbial Study of Pathogenic Bacterial that Producing Biofilm Isolated From Fresh Red Meat

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Abstract

Contamination of meats with different species of microorganisms pose significant threats not only to the human health but also to the animal production. This study was conducted to investigate the presence of bacterial contaminants in raw meat of livestock. A total of 75 random samples were collected (included 50 cow samples and 25 goats samples) from several butcher shops and slaughterhouse stores in Basrah governorate. The samples were subjected into the biochemical tests using Enter system 18R to confirm preliminary bacterial diagnosis and subsequently cultured using a selective media (MacConkey agar). The outcomes demonstrated *Escherichia. coli* was the dominate bacterial isolated species in the cow and goats with the percentage estimated at (30%) and (28%), respectively. Other important isolations were also found Enterobacter cloacae in was a rate (20%) Cow meat and *Klebsiella pneumonia* in goat meat with a rate estimated at (20%), while the lowest isolations rate was Salmonella spp (8%) in cow meat. *Staphylococcus aureus* (14%) in cow meat and (12%) in goat meat. In conclusion, meats are representative the source of infection with foodborne pathogens carry hazard to public health transmitted to the humans due to mishandling and improper hygienic condition of meats. The results showed biofilm production were high percent for *Staphylococcus aureus* 5 (50%) strong biofilm producer, follow *Klebsiella pneumonia* 4 (30.76%) strong biofilm. As other isolates were biofilm producer and production of biofilm is related to the resistance.

Keywards: Red meat, Biofilm , Bacterial pathogens

دراسة ميكروبية لمسببات الأمراض البكتيرية المنتجة للأغشية الحيوية المعزولة من اللحوم الحمراء الطازجة

الخلاصة

شكل تلوث اللحوم بأنواع مختلفة من الكائنات الحية الدقيقة تهديدات كبيرة ليس فقط على صحة الإنسان ولكن أيضًا على الإنتاج الحيواني. أجريت هذه الدراسة للتحقق من وجود الملوثات جرثومية في اللحوم الطازجة للماشية. تم جمع 75 عينة عشوائية (تتضمن 50 عينة من البقر و 25 عينة من الماعز) من عدة محلات جزارة في مناطق مختلفة من محافظة البصرة. تم إخضاع العينات للاختبارات الكيموحيوية باستخدام نظام Enter 18R لتأكيد التشخيص البكتيري الأولي ومن ثم تربيتها باستخدام الوسائط الانتقائية (MacConkey agar). وأظهرت نتائج الدراسة ان نسبة عزل جراثيم الاشريكيا الإشريكية من الأنواع الجرثومية المعزولة السائدة في الأبقار والماعز بنسبة عزلها تقدر بـ 30% و28% على التوالي. كما وجدت جراثيم هامة أخرى مثل Enterobacter cloacae و Klebsiella pneumonia في لحوم الأغنام بنسبة تقدر بـ 20%، في حين كانت أقل نسبة للعزلات هي جراثيم السالمونيلا و جراثيم المكورات العنقودية الذهبية بنسبة تقدر 8%. في الختام، تعتبر اللحوم مصدرًا للعدوى بمسببات الأمراض المنقولة بالغذاء والتي تحمل خطرًا على الصحة العامة التي تنتقل إلى البشر بسبب سوء التعامل والحالة الصحية غير السليمة للحوم. أظهرت النتائج أن إنتاج الأغشية الحيوية كان عالياً بالنسبة لجراثيم الكورات العنقودية الذهبية (50%) 5 المنتجة للأغشية الحيوية القوية، تليها (30.76%) 4 (Klebsiella pneumonia للأغشية الحيوية القوية. كما أن العزلات الأخرى كانت منتجة للأغشية الحيوية ويرتبط إنتاج الأغشية الحيوية بالمقاومة

Introduction

More recently, as human population have twice expanded and increased globally, meat has become an economically important (1). Besides, meat flash is considerably useful food sources for human supply that contains essential proteins and fats. Likely, the animal carcass after having been slaughtered may be exposed to a variety of bacterial contamination originated chiefly from mishandling of slaughtering animal process at the abattoirs (2).In addition to phosphate, vitamins, fat, water, protein, and iron, meat provides a nutrient-rich source of protein for people. Water is present in large amounts in the majority of the meat, which promotes the growth of microorganisms. meat tainted by a number of things, including the environment, human treatment, manipulation, and/or the animal itself. (3). Foodborne pathogens have ability to produce different types of enterotoxins and obviously are harmful to the human health through causing sever illness and mortality. Furthermore, proliferation and rapid growth of bacteria leads to double-increasing meat putrefaction because of a pH change of meat and spoilage of meat (4). Spoilage of meat depends on different circumstances which may be including the number of bacteria, time, and temperature combination of storage conditions and physicochemical properties of meat (5). Some of these bacteria when is badly storage, can survive under-optimum condition of temperature and humidity affected quality, spoilage meats and economic losses (6). However, the most common foodborne bacterial pathogens that related to meat contaminations are *Salmonella* spp., *Staphylococcus aureus*, *Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Clostridium perfringes*, *Yersinia enterocolitica* and *Aeromonas hydrophila* (7). These microorganisms, have a larger extent to human being noticeably in low-income countries where lack of hygienic awareness and absence of intensive food health inspection (8). Approximately, more than 1.5 million incidence cases have been reported annually infected with *Salmonella* spp, *Escherichia coli* (8). These bacteria cause serious complication and deterioration to the infected hosts like causing gastroenteritis, bloody diarrhoea, and haemolytic uremic syndrome (9).Over 80% of all bacterial illnesses are biofilm-contained, according to a National Health Institutes research. Health issues include urogenital infections, dental plaque, indwelling medical device infections, and upper respiratory tract infections are all linked to biofilms, in addition the antibiotic resistance might grow 1,000 times , these biofilms are very challenging to eliminate. A variety of methods, including tissue culture and microtiter plates, are available for the identification of biofilms.(25). Biofilms have been a significant contributing factor to pathogenicity. Additionally, biofilm-forming isolates with antibiotic resistance contribute to bacterial persistence, which can result in persistent infections and treatment issues (26). Little studies were done in Basrah to investigate on foodborne pathogens in meats. Therefore, this study was conducted to investigate on the bacteria isolated from the cow and goats meats sampled randomly from the butcher shops in Basrah Governorate.

Materials and Methods

Sampling

The period of this study was November to October from 2022 the butchery and slaughterhouse stores from different regions in Basrah governorate, of which included 75 samples with 50 cow meat and 25 goat samples. The samples were collected in sterile containers and immediately retrieved inside an ice box and

transferred to the laboratory for the bacteriological analysis.

Isolation of bacteria

All samples were treated aseptically, of meat were weighed and aseptically cut into thin smaller pieces by using sterile knife and then added to 225 ml of buffered peptone water. The inoculated media were incubated at 37 ° C for 18 hrs (10). The samples were cultured on blood agar and MacConkey agar to differentiate between the lactose and non-lactose fermenting bacteria.

Identification of bacteria

Isolates of bacteria identified by subculturing in MacConky agar to distinguish between the lactose and non-lactose fermenting and then the isolates were submitted to identified by Enterosystem 18 R.

Biofilm formation assay

Cellular adhesion to the wells of a 96-well microtiter plate was used as a phenotypic indicator of biofilm formation. In order to remove any unattached cells, 200 microliters of this bacterial culture were used to inoculate 96-well polystyrene microtiter plates that had already been cleaned and sterilized. After 48 hours at 37°C of incubation, all wells were then rinsed with sterile physiological saline. To each well, 200 l of 1% crystal violet was then added. Each well rinsed with 200 of sterile physiological saline following 15 min. at room temperature. There were three iterations of this process. An ELISA reader was used to measure absorbance at 540 nm after the crystal violet that was bound to the biofilm was later removed with 200 l of ethyl alcohol (26).

Statistical analysis

The results obtained using SPSS were analyzed using Chi-square at the level of significance ($p < 0.05$), (30)

Results and Discussion

The results of this study showed the presence of bacterial contamination with six bacterial genera in identified in the swab meats belong to cows and goats. There is no significant difference in bacterial species ($p < 0.05$), shown in tables (1) and figure (2). The hygienic condition of the environment butcher shops and its slaughter tools are more likely contributed for the contamination of meat. The biochemical test and media culture showed the contamination of cow with E. coli spores with the highest percentage estimated30% of cow samples and 28% in goat meat In contrast, the studies were performed by showed the isolation rate with E.coli found to be 80% from a minced cow (11). This maybe because minced meat is more susceptible to according to report published by ACT Health (12), contamination of fresh meat reached 16.6%, and the insulation ratio mentioned by it (13) reached 19% as fuel due to rinsing of slaughter after the removal of entrails (14). As well as "the small numbers of beef carcasses, the season may be one of the factors affecting the rate of contamination, as increased contamination of the carcasses in the rainy season (15). The Salmonella bacterium was isolated from cow by 8%, and this corresponded to (16), as the isolation ratio was 8% While the Salmonella bacterium was isolated from goats meat with a rate of isolation of 16%, and compared to local studies, the contamination rate shown by this study is higher than the isolation rate found by each of (17) in goats carcasses in Mosul and (18) in goats meat in Baghdad, and the rate of isolation in Mosul from goats meat amounted to 7% (19) and the percentage mentioned by it (20) in goats meat in Anbar Governorate is identical, as the isolation rate is 4.9% for salmonella germ compared to the percentage mentioned by (18) which reached 5.5% for goats carcasses in the Madras massacre in India It is more than the

percentage of isolation mentioned by (17), as it reached 1.3% in goats carcasses in Australia, that the difference due to isolation between . The various governorates of Iraq and among the different countries of the world indicates the extent to which the health conditions are applied (21).The results showed the isolation of Klebsiella pneumonia from cows meat 16%, which is less than the isolation rate that it found (22), reaching 21.5% in cow meat, (23) indicated the rapid spread of Klebiella spp. bacteria in meat and meat products due to unhealthy handling and poor storage conditions. The high incidence of contamination of goats carcasses with some bacterial species than in cow carcasses is due to the different nature of goats meat fibers than cows, since the penetration of bacteria for goats meat is faster than the penetration of bacteria for cow meat, that the occurrence of food poisoning by eating beef is less frequent (24).

Most bacterial isolates were produce biofilm .while was all *Enterobcter cloacae* isolates produce biofilm (100%). 6 (42.85) moderate biofilm and 8 (57.14) strong biofilm . *Staphylococcus aureus* isolates were 5 (50%) strong biofilm. Also *Klebsiella pneumonia* and *Salmonella spp* were non- producing strong biofilm. in *Escherichia coli* was 9(40.9) strong biofilm and 1(4.54) non-produce biofilm. who mentioned that the differences in of forming-biofilm were no significantly between the bacterial species ($p < 0.05$), table 2, Fig 3. The current study found all isolates were production of biofilm was 6 / 42.85 moderate biofilm and 8 / 57.14 strong biofilm for *Enterobcter cloacae* this result in line with the result of in Baghdad - iraq who showed presence (60 %) were strong biofilm producer and (40 %) of all isolates were moderate biofilm producer (25) . In Escherichia coli isolates were able to produce strong biofilm (40.9%).while the moderate biofilm was 22.72% and weak biofilm 31.81% . in *Klebsiella pneumona* isolates produce moderate biofilm 61.53% and weak biofilm 38.46% this study consistent with results of previous study by (26) in Basrah-Iraq.in Proteus mirabilis produce biofilm were Result in this study is an agreement with a result of a study done by (27) in Kirkuk- Iraq. Also salmonella spp. Isolates forming biofilm 50% moderate biofilm and 25% was weak biofilm in this study but was differ from the result reported in Basrah-Iraq by (28). In this study a high percentage for Staphylococcus aureus biofilm producing 50% strong biofilm and 20% moderate and 20% non-biofilm. This study agreement with a result of a previous study (29).

Figure (1): The biochemical positive tests results for the precise identification bacteria

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Table 1: shows the genera and the percentage of bacterial isolates from the fresh red meat (cow and goat)

Bacteria type	Cow meat		Goat meat	
	Isolation No.	No. percent	Isolation No.	No. percent
Enterobcter cloacae	10	20 %	4	16%
Escherichia coli	15	30%	7	28%
Klebsiella pneumonia	8	16%	5	20%
Proteus mirabilis	6	12%	2	8%
Salmonella spp.	4	8	4	16
Staphylococcus aureus	7	14	3	12
Total	50		25	
	Chi-Square ($\chi 2$)	0.56	966**	
** (P≤0.01), Non-Significant.				

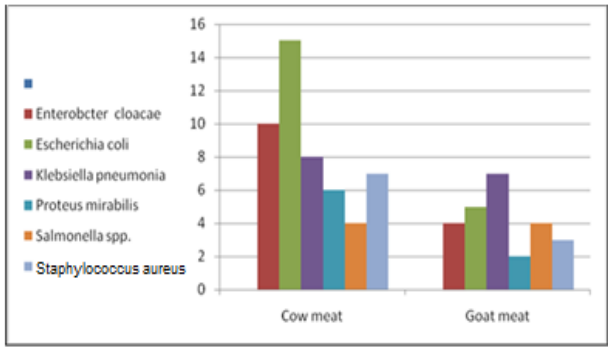

Figure (2) shows the number and types of isolates in soft meat

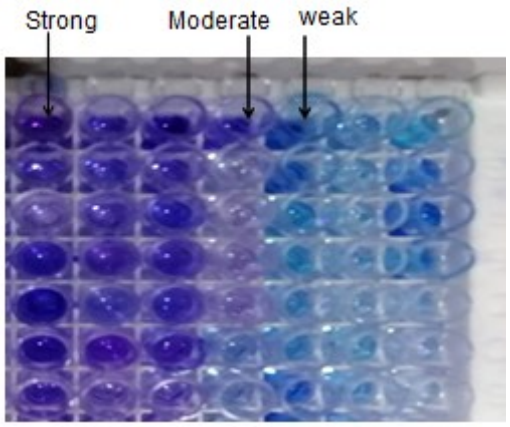

Figure 3 :pre-sterilized 96-well polystyrene microtiter plates biofilm production.

Table 2: Number and percent for different bacterial species of forming-biofilm of isolated each from cow and goat meat

Bacterial Species	Not Biofilm Producer No. (%)	Weak Biofilm Producer No. (%)	Moderate Biofilm Producer No. (%)	Strong Biofilm Producer No. (%)
Enterobcter cloacae	0	0	6 / 42.85	8 / 57.14
Escherichia coli	1/ 4.54	7 / 31.81	5 / 22.72	9/40.9
Klebsiella pneumonia	0	5/38.46	8/61.53	0
Proteus mirabilis	1/ 12.5	2 / 25	3/ 37.5	2/25
Salmonella spp.	2/25	2/25	4/50	0
Staphylococcus aureus	2/ 20	1/10	2/20	5/ 50
Total	6	18	32	19
	Chi-Square ($\chi 2$)	18.82	0.92**	
** (P≤0.01), Non-Significant.				

There is significant difference at the level of significance (P<0.05).

Conclusion

The difference in the isolation ratios of the different bacterial races may be due to the extent of application of the health conditions used in each place and the nature and intensity

of production and according to the development of the country we have legalized or underdeveloped through this study, we have noticed the underdeveloped reality of places of slaughter and their distance from the simplest health ingredients. According to our knowledge in this is study in IRAQ used which showed a biofilm formation for most of bacteria isolated from red meat and this mean most the isolates have the resistance against antibacterial activity.

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Conflict of interest

Declare that there are no conflicts of interest regarding the publication of this manuscript.

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