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RESEARCH ARTICLE

Evaluation of Hygienic Status of Some Meat Products Retailed in Zagazig City, Egypt

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ABSTRACT

This study aimed to assess the underexplored hygienic status of marketed meat products (minced meat, sausage, and luncheon) in Zagazig city, Sharkia Governorate, Egypt. Sixty meat product samples from different hypermarkets were analyzed for aerobic plate count (APC), Psychrotrophic bacteria count (PBC), coliforms, S. aureus and mold counts. The prevalence was 100% for APC, and PBC in all examined meat product samples, 100% for coliforms in minced meat, and sausage and 50% in luncheon samples, 80%, 90%, and 60% respectively for S. aureus in the examined meat product samples, meanwhile 100% for mold in all meat product samples. The mean counts of APC (4.73±0.21, 5.25±0.21, and 4.06±0.17 log10 CFU/g), PBC (4.35±0.20, 4.90±0.21, and 3.57±0.10 log10 CFU/g), and S. aureus (2.74±0.11, 3.01±0.13, and 2.42±0.14 log10 CFU/g) were statistically different ($P < 0.05$) in all tested meat products. Similarly, the counts for coliform and mold showed significant differences between the three types of meat products. This suggests an increased risk of spoilage and pathogen contamination. In conclusion, the obtained data demonstrated that the hygienic precautions that were implemented during the manufacturing of such products were insufficient. Therefore, there is an immediate and pressing requirement for improved hygienic standards in meat processing factories and supermarkets in order to limit the amount of microbial contamination and protect the safety of consumers.

Introduction

Meat serves as an important source of high-quality protein and bioavailable vitamins, along with essential minerals like iron, zinc, and phosphorus [1]. Meat products are gaining popularity as a result of the fact that they can be easily and quickly prepared into meat meals, which can help in addressing the issue of a shortage of fresh meat that is extremely expensive [2].

One of the most major problems that affects the quality of meat, and the health of the general public is the presence of microbiological contamination. It is possible that the microbiological quality of beef products is impacted by a variety of factors, such as the lengthy chain of preparation, processing, distribution, storage, and retailing [3].

A number of different methods, including aerobic plate count (APC), total psychrotrophic counts, total *Enterobacteriaceae* counts, total coliforms, *Staphylococcus*, and mold, can be utilized to assess the level of bacterial contamination and sanitary measures that are implemented during the manufacture of meat as well as negative storage conditions for meat products [4-6]. The APC is a significant measure of the sanitary quality of a product throughout the entire process of processing and distribution [7]. The APC level in meat is regulated or recommended by a number of countries, which allows them to control the meat hygiene. Within the year 1978, the International Commission on Microbiological Specifications for Foods (ICMSF) was the sole entity that established the APC guideline level, which was set at 1.0 × 10^7 CFU/g or cm^2 [8]. The permissible limits for total bacterial count in meat products vary depending on the type of product and its processing. Generally, raw meat products have higher acceptable limits than cooked or processed products. For example, raw poultry products may have a limit not exceeding 10^5 CFU/g, while heat-treated products might have a limit of 10^4 CFU/g [9]. As a result of their capacity to thrive at low temperatures, psychrotrophic bacteria are the primary culprits responsible for the deterioration of meat products that are stored at temperatures that are below the refrigeration threshold [10]. There is a correlation between high counts of psychrotrophic bacteria and an environment that is conducive to the development of illnesses [11].

Psychrotrophic bacteria are excellent markers of the hygienic state of meats at low temperatures and their potential for spoilage. Coliforms are types of bacteria that are found in the stomach, and the number of them can be used as a measure of hygienic conditions [12]. The permissible limits for total psychrotrophic counts in meat products vary depending on the type of product and regulations, but generally, raw poultry products should not exceed 10^5 CFU/g, and heat-treated products should be below 10^4 CFU/g [13].

Escherichia coli (*E. coli*) is a Gram-negative, facultative, anaerobic bacteria that is regarded to be a commensal organism in the human body [14]. Consumers may experience food poisoning if proper cooking procedures are not followed [15]. As of right now, *E. coli* isolates that cause diarrhea are divided into six primary pathotypes. These pathotypes are distinguished from one another by their unique virulence factors and pathogenic characteristics [14]. The shiga toxin producing *E. coli* (STEC) group is considered to be one of the most significant pathotypes associated with human infectious diseases. Raw meat should also have acceptable levels of coliform bacteria, with some standards specifying limits of 10^2 CFU/g. Cooked or processed meats generally have stricter limits, often around 10^4 CFU/g for APC [15].

Staphylococcus aureus (*S. aureus*) is a known opportunistic pathogen that is spread through food and has been linked to multiple outbreaks in hospitals and communities worldwide [16]. Furthermore, *S. aureus* frequently colonizes human skin and can lead to opportunistic infections in the host, which can contaminate or re-contaminate cooked meals through the hands, tools, or utensils of workers [17]. The acceptable limit for *S. aureus* count in meat products is generally less than 100 CFU/g according to the International Microbiological Criteria [18].

Mold is considered one of the causative agents of meat deterioration; it is responsible for the world's economic losses from food's production by 5-10% [19]. Meat products contaminated by mold were a sign of unhygienic and unsanitary handling, processing, and storage conditions [4]. Heat-treated meat products should be free from molds [20].

In light of the previous facts, the current study was carried out with the purpose of assessing the microbiological condition of a selection of meat products including minced meat, sausage, and luncheon in Zagazig city. This was accomplished through the assessment of APC, mold, coliform, *S. aureus* and psycrotrophic bacterial counts.

Materials and methods

This study was conducted in accordance with Zagazig University's procedures, and no living animals were used.

Samples collection and preparation

A total of sixty samples of meat products including minced meat, sausage, and luncheon, 20 each were obtained randomly from a variety of stores in Zagazig city, Sharkia Governorate, Egypt. For the purpose of conducting a microbiological analysis, the samples were without delay and delivered to the Meat Hygiene Laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Microbiological analyses

In accordance with the methodology outlined in ISO 6887-2 [21], samples of meat products and serial dilutions were created. In summary, twenty-five grams of each sample were meticulously blended using a sterile homogenizer flask (Homogenizer type MPW–302, Poland) containing 225 milliliters of sterile peptone water (Hi media, India) diluted to a 0.1% concentration. A 1/10 dilution homogenate was produced by homogenizing the contents for 2.5 minutes at 5000 revolutions per minute. The homogenate was permitted to rest for roughly 5 minutes. Following the transfer of the homogenate into a sterile test tube containing nine milliliters of one percent peptone water, tenfold serial dilutions were generated, achieving a concentration of 10^6 per milliliter.

The following analytical procedures were carried out on the samples that had been prepared: In accordance with ISO 4833-1 [22], the APC was carried out by employing a sterile plate count agar and inoculating it at 37 °C for 24 hours. On plates that contained between 30 and 300 colonies, the APC computed per gram was determined.

For determination of the psychrotrophic counts [23], accurately 0.1 mL from each of the previously prepared serial dilution was spread over a dry surface of a plate count agar medium (Oxoid, UK) by a sterile bent glass spreader. The plates were incubated at 7 °C for 10 days together with the control plates. All colonies were counted, the average number of colonies was recorded, and the total psychrotrophic bacterial count (CFU/g) was calculated.

After incubating the samples at 37 °C for 24-48 hours, the most probable number (MPN) of coliforms was determined by counting the number of coliforms that were incubated in three MacConkey broth tubes with inverted Durham's tube. It was determined that the generation of acid and gas in the inverted Durham's tubes was found to be positive and evaluated in accordance with the ICMSF [24].

The count of *S. aureus* was carried out using each of the serial dilutions that had been produced in advance. Using a sterilized glass spreader, 0.1 mL from previously prepared serial dilutions was evenly distributed on Baired Parker agar plates. The plates were incubated at 37 ˚C for 48 hours. The shiny black colonies surrounded by a halo zone were counted per gram of sample [25].

After inoculating the agar at a temperature of 25 ˚C for five days, the total mold count was determined by using Dichloran Rose Bengal Chloramphenicol agar (Oxoid, UK) that contained chloramphenicol at a concentration of 0.05 mg/mL [26]. A "star-shape" mold growth was counted and recorded as the total mold count per gram of sample.

Statistical analysis

Microbial counts were transformed into their logarithmic values (Log_{10} CFU/g). One-way analysis was performed on the data that was acquired using the SPSS 16.0 program (SPSS Inc., Chicago, Illinois, United States of America). In order to determine whether or not there were significant differences between the mean values, the Tukey's multiple comparison tests were utilized. The results were denoted as the mean plus the standard error (SE), and a significance level of $P < 0.05$ was utilized to determine the statistical significance [27].

Results

In Table 1, the findings of the prevalence analysis of APC, psychrotrophic, coliform, *S. aureus*, and mold contamination in sixty samples are presented.
The results indicated that 100% of all examined meat products were positive for APC, psychrotrophic bacteria, and coliforms (except 50% for luncheon). It is important to note that both APC and PBC were positive for all examined meat products, which indicated that aerobic and psychrotrophic bacteria were present in rather high concentrations. Coliform detection was high in samples of minced beef and sausage (100%), but it was low in samples of luncheon (50%). Furthermore, the prevalence of *S. aureus* was found to be slightly greater in sausage samples (90%) compared to minced meat (80%), while it was found to be lower in luncheon samples (60%). All tested meat product samples were positive to molds (100%).

The results demonstrated in Table 2 revealed that the minimum and maximum APC were ranged from 3.79 to 5.71, 4.46, to 6.18, and 3.28 to 4.79 Log_{10} CFU/g in minced meat, sausage and luncheon respectively. The minimum and maximum psychrotrophic were fluctuated from 3.45 to 5.38, 3.99 to 5.85 and 3.04 to 3.96 Log_{10} CFU/g in minced meat, sausage and luncheon, respectively. The minimum and maximum coliforms were ranged from 2.04 to 3.79, 2.18 to 4.18 and 2.66 to 3.18 Log_{10} CFU/g in the three examined meat products, respectively. Meanwhile, for *S. aureus* such values ranged from 2.30 to 3.30, 2.60 to 3.78 and 2.00 to 2.85 Log_{10} CFU/g for minced meat, sausage and luncheon, respectively. The minimum and maximum total mold count was varied from1.85 to 2.70, 2.00 to 3.30 and 2.00 to 3.60 Log_{10} CFU/g in minced meat, sausage and luncheon, respectively.

The results of mean counts (Table 2) illustrated that the highest APC, PBC, coliforms, *S. aureus*, and mold counts were recorded in sausage samples (5.25±0.21, 4.90±0.21, 3.35±0.22, 3.01±0.13, 2.73±0.20 log_{10} CFU/g, respectively) and the lowest counts were obtained in minced meat for APC (4.73±0.21 log_{10} CFU/g), luncheon for PBC (3.57±0.10), coliforms (2.92±0.09) and *S. aureus* (2.42±0.14 log_{10} CFU/g), respectively, and minced meat for mold (2.22±0.18 log_{10} CFU/g).

Table 1: Prevalence of aerobic plate count, psychrotrophic bacteria count, coliform, *S. aureus* and mold counts in different types of meat product samples (no=60).

Types of meat products	Microbial agents	Positive Samples	
		No.	%
Minced meat	**APC**	20	100
	PBC	20	100
	Coliforms	20	100
	S. aureus	16	80
	Mold	20	100
Sausage	**APC**	20	100
	PBC	20	100
	Coliforms	20	100
	S. aureus	18	90
	Mold	20	100
Luncheon	**APC**	20	100
	PBC	20	100
	Coliforms	10	50
	S. aureus	12	60
	Mold	20	100

No, Number of samples; APC, Aerobic plate count; PBC, Psychrotrophic bacteria count; *S. aureus*, Staphylococcus aureus.

Table 2: Statistical analytical results (log_{10} CFU/g) of aerobic plate count, psychrotrophic bacteria count, coliform, *S. aureus* and mold counts in examined meat products samples (n=20 for each meat product).

Types of meat products	Microbial agents	Microbial count		
		Minimum (Log_{10} CFU/g)	Maximum (Log_{10} CFU/g)	Mean ±SE
Minced meat	APC	3.79	5.71	4.73±0.21 ^{b}
	PBC	3.45	5.38	4.35±0.20 ^{b}
	Coliforms	2.04	3.79	3.04±0.18 ^{a}
	S. aureus	2.30	3.30	2.74±0.11 ^{ab}
	Mold	1.85	2.70	2.22±0.18^{b}
Sausage	APC	4.46	6.18	5.25±0.21 ^{a}
	PBC	3.99	5.85	4.90±0.21 ^{a}
	Coliforms	2.18	4.18	3.35±0.22^{a}
	S. aureus	2.60	3.78	3.01±0.13 ^{a}
	Mold	2.00	3.30	2.73±0.20^{a}
Luncheon	APC	3.28	4.79	4.06±0.17 ^{c}
	PBC	3.04	3.96	3.57±0.10 ^{c}
	Coliforms	2.66	3.18	2.92±0.09^{a}
	S. aureus	2.00	2.85	2.42±0.14 ^{b}
	Mold	2.00	3.60	2.83±0.19^{a}

^{a,b,c} Means carrying different superscript letters within each microbial category are significantly different at $P < 0.05$ among the examined minced meat, sausage, and luncheon. No, Number of samples; APC, Aerobic plate count; PBC, Psychrotrophic bacteria count; *S. aureus*, *Staphylococcus aureus*.

Discussion

Meat products such as minced meat, luncheon, and sausage may be contaminated at any stage of the processing, packing, and shipping processes with various pathogens. The meat products become hazardous to customers and inappropriate for human consumption due to these pathogens. The hygienic status of these products can be assessed using a variety of indicators; Coliforms are frequently used to assess the safety and cleanliness of meat products [9, 28].

The microbiological quality and safety of meat in any country requires continual and regular monitoring and updating. It is also necessary for the industry to ensure that consumers are provided with meat and meat products that are of high quality and safe for consumption [29].

Aerobic plate count (APC), PBC, coliforms, *Staphylococci*, and mold counts were conducted in this study in order to evaluate the microbiological quality of meat product samples. These samples included minced meat, sausage, and luncheon. All of the samples demonstrated high levels of APC, with the mean values being 4.73±0.21, 5.25±0.21, and 4.06±0.17 log_{10} CFU/g for the minced beef, sausage, and luncheon samples, respectively. It is of the utmost importance to evaluate the hygienic conditions under which a food item has been created, handled, and stored [30]. Although the APC of any food item is not a definitive indicator of whether or not it is safe for ingestion, APC is frequently utilized to evaluate the overall microbiological quality of meat as well as its shelf life [31, 32]. It additionally provides an indication of the hygienic quality of a product throughout the entire process of processing and distribution [33].

The findings that were presented in Table 2 demonstrated that the APC results in minced meat agreed with the findings that were presented by Erdem-Ayten *et al.* [34]. On the other hand, researchers found lower levels in minced meat [35], as well as in sausage and luncheon [36]. However, minced meats had higher microbial counts in previous research [37]. Furthermore, aforementioned study indicated that the mean values (log_{10} CFU/g) of the APC/g were 6.1±0.1 in minced meat, 4.8±0.1 in sausage, and 4.2±.1 in luncheon [38]. A high APC could be the result of the product being contaminated from a variety of sources, or it could be the result of processing that was not adequate, or it could be the result of storage conditions that were not suitable [39].

As a result of their capacity to thrive at low temperatures, psychotrophic bacteria are the primary culprits responsible for the spoilage of meat products that are stored at refrigeration temperatures. It is possible to obtain valuable information regarding the preservation quality of certain meat products by utilizing the total PBC [5]. According to the current investigation, the presence of PBC was detected in all samples, with significant variations observed ($P < 0.05$). The mean value of their counts in the samples of sausage was 4.90±0.21 log_{10} CFU/g as well as minced meat (4.35±0.20 log_{10} CFU/g) and luncheon samples (3.57±0.10 log10 CFU/g). In this study, the obtained minimum and maximum values of the total psychotrophic count (log_{10} CFU/g) in the meat samples (minced meat, sausage, and luncheon) were nearly similar to other investigations that were conducted previously [40-44]. The latter reports showed these results were more favorable. The presence of high PBCs in this study may be an indication of inappropriate hygienic practices during slaughtering, processing, or retail handling [11]. Psychrotrophic bacteria are able to thrive in cold environments because they have developed adaptations that allow them to do that. These adaptations include proteins that are able to function at low temperatures and increased quantities of unsaturated fatty acids in their cell membranes. Despite the fact that their presence in food might cause it to go bad or put people at danger of getting sick, they also play an important part in the process of biodegradation in natural ecosystems, particularly during the winter months [45]. On the other hand, our findings regarding PBCs seemed to be in direct opposition to those obtained by previous researchers [5, 40].

There is no evidence that coliform bacteria are the major cause of spoilage; rather, their presence is indicative of insufficient hygiene or the possibility of cross-contamination during the preparation or storage of the food [11]. Regarding the evaluation of coliforms in this study, the findings indicated that the presence of coliforms was more abundant in the samples of minced meat and sausage (100%) compared to the samples of luncheon (50%) with mean values of 3.04±0.18, 3.35±0.22, and 2.92±0.09 $\log_{10}$ CFU/g, respectively. Similar findings were obtained by previous researchers [38, 46-50]. Coliform counts` results in this investigation were 3.04±0.18, 3.35±0.22, and 2.92±0.09 $\log_{10}$ CFU/g in minced meat, sausage, and luncheon, respectively. When compared to the results found in minced meat (2.80±0.10 $\log_{10}$ CFU/g) [35], luncheon and sausage (3.1 and 3 $\log_{10}$ CFU/g respectively) [33], these findings were almost identical to those seen in minced meat. On the other hand, higher results were obtained in minced meat (4.5 x 107 log10 CFU/g) [34]. Furthermore, the mean values of coliforms expressed as $\log_{10}$ CFU/g was found to be 2.6±0.1 in luncheon, 3.1±0.1 in minced meat, and 2.9±.01 in sausage, as reported elsewhere [9]. Furthermore, the statistical findings demonstrated that there is no significant difference (P > 0.05) in the coliform counts between the samples of meat products (minced meat, sausage, and luncheon). It is possible that the nature of coliforms, which may not flourish as efficiently under cold storage conditions as psychrotrophic bacteria, is the reason for lower coliform counts as compared to APC and PBC. Coliforms are a specialized subset that comprise a lesser fraction of the entire microbial community in meat [11].

The data that were obtained and presented in Table 2 showed that the minimum and maximum counts ($\log_{10}$ CFU/g) of *S. aureus* in the investigated meat products varied from 2.30 to 3.30 in minced meat, 2.60 to 3.78 in sausage, and 2.0 to 2.85 in luncheon, which are consistent with the findings of previous studies [48, 51, 52]. Likely, Shaltout *et al.* [9] observed that the mean values ($\log_{10}$ CFU/g) of *S. aureus* counts/g were 2.2 ±.1 in minced meat, 2.6 ±.1 in sausage, and 2.5 ±.1 in luncheon. Furthermore, Shaltout *et al.* [5] and Younis *et al.* [53] obtained data that were comparable to the examined luncheon samples. Both of these studies were conducted on minced meat. *S. aureus* count was found to be the highest in samples of sausage (3.01±0.13), followed by mined meat (2.74±0.11), while the lowest level was found in luncheon (2.42±0.14). It is possible that the reason for the high *S. aureus* counts is the fact that the majority of markets are typical open-air marketplaces that do not have air conditioning; these factors enhance the likelihood of bacterial contamination and subsequent growth [54]. In the meantime, lower results were recorded by several writers [43, 55]. On the other hand, S. aureus had not been detected in minced meat as reported by other studies [56, 57].

Mold contaminations of meat and meat products can occur during the slaughtering of animals, transportation, or processing of meat products through the use of contaminated equipment or contaminated additives and spices, which are considered to be the most significant source of contamination in meat products [58]. Mold was found in every single sample of meat product that was examined, without exception. As shown in Table 2, the total mold count (log10

CFU/g) ranged from 1.85 to 2.70 for minced meat, 2.00 to 3.30 for sausage, and 2.00 to 3.60 for luncheon. A slightly elevated count of mold was seen in the luncheon samples (2.83±0.19 log_{10} CFU/g) and sausage (2.73±0.20 log_{10} CFU/g), in contrast to the samples of minced meat, which had the lowest count (2.22±0.18 log_{10} CFU/g). Mold count results from luncheon samples were essentially identical to those obtained by other reporters [4, 59-61]. On the other hand, a higher prevalence of mold was recorded in meat samples [62, 63]. The results of sausage samples were nearly similar to those obtained previously [60, 64] and were significantly lower than those obtained by Brr et al. [65]. Furthermore, the obtained results in this study are nearly similar to those obtained by Saad et al. [66] and were lower than those reported previously [63, 67].

It is possible that the fluctuations in the overall number of molds found in samples are the result of varying degrees of cleanliness during the manufacturing and storage processes. The highest total mold count in luncheon may be related to poor handling or storage circumstances, whereas the mold count in sausage may be attributed to the composition of sausage, which is minced meat packed in the intestines of animals that may not have been sufficiently cleansed [4].

Conclusion

The findings of this research showed that the meat product samples that were analyzed (minced meat, sausage, and luncheon) were substantially contaminated with a variety of different species of microorganisms. This contamination was a result of faulty sanitary practices and an inadequate environmental state that occurred throughout the handling, transportation, processing, and storage of the materials. APC and psychrotrophic species are the most prevalent types of bacteria that were found in the investigated samples. As a result, it is imperative that stringent hygiene procedures be adhered to throughout the processing and storage stages. In addition, stringent laws ought to be enacted in order to encourage the production of goods that have excellent preservation qualities.

Conflict of interest: None

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الملخص العربى

تقييم الوضع الصحي لبعض منتجات اللحوم المسوقة بمدينة الزقازيق – جمهورية مصر العربية

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تكتسب منتجات اللحوم شعبية متزايدة لأنها تُمثل وجبات سريعة وسهلة التحضير وبأسعار منخفضة، وتُمكّن المُصنّعين من تحويل أنواع اللحوم المختلفة إلى منتجات موحدة. يُعدّ تحديد الجودة الميكروبية وسلامة اللحوم أمرًا بالغ الأهمية نظرًا لاحتمالية احتوائها على مسببات الأمراض. هدفت هذه الدراسة إلى تقييم الحالة الصحية غير المستكشفة لمنتجات اللحوم المُسوّقة (اللحم المفروم، والسجق، واللانشون) في مدينة الزقازيق، محافظة الشرقية، مصر.

أجريت هذة الدراسة على عدد 60 عينة عشوائية من منتجات اللحوم (اللحوم المفرومة- السجق-اللانشون) وأجرى عليها التحليل البكتريولوجى حيث تم عزل وتعريف بعض مجموعات البكتيريا الممرضة وذات الخطورة على الصحة العامة مثل عدد الصفائح الهوائية (APC)، وعدد البكتيريا الباردة (PBC)، وعدد البكتيريا القولونية، والمكورات العنقودية، والعفن. كان معدل انتشار APC و PBC 100% في جميع عينات منتجات اللحوم المفحوصة، و 100% للكوليفورم في اللحوم المفرومة والسجق و 50% في عينات اللانشون، و 80% و 90% و 60% على التوالي للمكورات العنقودية الذهبية في عينات منتجات اللحوم، في حين كان معدل انتشار العفن في اللحوم المفرومة والسجق 100% و 50% في عينات اللانشون. كان متوسط تعداد البكتيريا المعوية (APC) 4.73±0.21، 5.25±0.21، 4.06±0.17 لوغاريتم/غرام)، وبكتيريا المكورات العنقودية الذهبية (4.35±0.20 (PBC، 4.90±0.21، 3.57±0.10 لوغاريتم/غرام)، والمكورات العنقودية الذهبية (0.11±2.74، 0.13±3.01، 0.14±2.42 لوغاريتم/غرام) مختلفًا إحصائيًا (P<0.05) في جميع منتجات اللحوم المختبرة، كما أن تعداد البكتيريا القولونية والعفن أظهر تغيرات ذات دلالة إحصائية بين ثلاثة أنواع من عينات منتجات اللحوم. وهذا يشير إلى زيادة خطر التلف والتلوث بمسببات الأمراض. كشفت النتائج عن قصور في إجراءات النظافة أثناء نقل ومعالجة وتخزين وتوزيع اللحوم ومنتجاتها. وهذا يُبرز الحاجة المُلحة لتحسين ممارسات النظافة في المتاجر الكبرى للحد من التلوث الميكروبي وضمان سلامة المستهلك.