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Assessment of Microbiological Quality of Imported Meat Products in Yemeni markets with Focus on *Pseudomonas* spp. as a Spoilage Indicator

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Abstract

Meat products are very perishable foodstuff that can easily be contaminated by microbial organisms and spoiled due poor storage and handling facilities. The current research was conducted to determine microbiological quality of imported meat products in Yemeni retail market with emphasis on use of *Pseudomonas* spp. to test the level of spoilage. A total of 214 samples that included beef burgers, chicken burgers, chicken meat, minced beef and minced lamb were aseptically collected at the 2 supermarkets in Sanaa in Yemen. Each sample was analyzed by standard culture-based procedures to achieve total aerobic plate count (APC), and presence of *Pseudomonas* spp., followed by species-level identification with the help of VITEK(R) 2 Compact system. The number of minced beef samples and minced lamb samples had the highest mean APC values of 8.87 10 5 and 8.55 10 5 trend, respectively, lower values were found in burger samples and chicken meat samples. The percentage rates of detection of *Pseudomonas* spp. were 16.36 with the maximum percentages found in minced lamb (35.29) and minced beef (25.00). *Pseudomonas aeruginosa* (60ATTR Morphological identification of *Pseudomonas* spp. showed that *P. aeruginosa* was the commonest isolate (60%) followed by *P. fluorescens* (40%). The samples were all compliant with the Yemeni GSO 1016:2019 and 83.64 percent with the ISO 13720:2010, which states that the microorganisms must not have *Pseudomonas* spp. Such results support the increased susceptibility of minced meats towards microbial contamination and putrifaction. In revising this study, the researchers suggest that they put *Pseudomonas* spp. monitoring as part of the national food safety policy and strengthening the cold chain to increase meat quality control and safeguard the health of the people in Yemen.

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1. INTRODUCTION

Meat and meat products are vital components of the human diet, offering high-quality protein, essential amino acids, vitamins, and minerals [1] However, these products are highly perishable because of their nutrient-rich composition, high water activity, and near-neutral pH, which create optimal conditions for microbial growth and spoilage [1–3]. Microbial contamination can occur at various stages of meat production slaughter, processing, transport, and retail particularly in the absence of adequate hygiene measures [4, 5]. Therefore, strict hygiene practices and quality control are essential throughout the supply chain to minimize contamination risk [6, 7].

spoilage of meat is primarily associated with psychrotrophic microorganisms that proliferate under refrigeration. Among these, *Pseudomonas* spp. are predominant because of their ability to grow aerobically at low temperatures [8–10]. These bacteria cause characteristic spoilage signs, such as slime formation, discoloration, off-odors, and off-flavors, largely through the production of sulfur-containing volatiles, esters, and ketones [9, 11]. While the total aerobic bacterial count (APC) is a widely used indicator of hygienic quality [12, 13] , it may not adequately detect early spoilage or

specific psychrotrophs, such as *Pseudomonas.*

Pseudomonas spp. are metabolically versatile gram-negative rods, well known for their role in meat spoilage and ability to withstand environmental stress and certain antibiotics [11, 14]. Recent studies conducted in Egypt, Iraq, and Vietnam have highlighted the frequent occurrence of Pseudomonas in both raw and processed meats, underscoring their relevance as spoilage indicators [15, 16]. Of particular concern is P. *aeruginosa*, which is associated with both a high spoilage potential and antimicrobial resistance [14].

Several countries in the MENA region continue to face serious public health challenges, particularly in contexts characterized by political instability and fragile healthcare infrastructure. Yemen stands out in this regard, with recurrent outbreaks of infectious diseases reported across different population groups [17–20]. These trends indicate systemic weaknesses in national surveillance and healthcare delivery. In addition, widespread concerns regarding food safety persist. Microbiological assessments of food products in Yemen have revealed elevated contamination levels, reflecting inadequate hygiene practices and weak enforcement within the food regulatory system [21]. These vulnerabilities increase the risk of foodborne illnesses caused by pathogens, such as *Campylobacter jejuni*, particularly in poultry. A recent systematic review analyzing data from 2014 to 2025 confirmed the presence of C. *jejuni* in chicken populations across Yemen and neighboring countries, highlighting a broader regional concern regarding zoonotic transmission through food [22].

In Yemen, food safety oversight is further compromised by ongoing conflicts, which disrupt cold chain logistics and inspection systems. With annual meat imports exceeding 40,000 tons, these products are especially vulnerable to microbial deterioration owing to frequent power outages and poor refrigeration monitoring [21, 22]. Compounding this issue is the absence of national standards that require testing for *Pseudomonas* spp., unlike international guidelines such as ISO 13720:2010, which emphasize their role in spoilage detection [11, 23].

In addition to food safety concerns, microbial spoilage of meat has significant economic consequences. This shortens shelf life, increases product returns, and undermines consumer trust. Global losses due to meat spoilage are substantial, particularly in settings with limited preservation infrastructure. In developed markets, even quality issues such as discoloration can result in multibillion-dollar annual losses [24],[25]

Given the limited availability of local data, this study aimed to evaluate the microbiological quality of imported meat products in the Yemeni markets, focusing specifically on *Pseudomonas* spp. as spoilage indicators. By identifying contamination patterns and characterizing isolated species, this study seeks to provide national policy updates and support the enhancement of food safety practices.

2. MATERIALS AND METHODS

2.1. Sample Collection

This cross-sectional study was conducted in Sana'a City, Yemen, between May and December 2024. A total of 214 random samples of imported poultry and other meat products were collected from the large supermarkets. All the samples were transported in insulated containers with ice packs at 4 ℃ and processed within two hours of collection. The countries of origin for these products are Saudi Arabia, Oman, the United Arab Emirates, and Brazil. The samples included 82 chicken, 61 minced (beef and lamb), and 71 burger samples (chicken meat and beef). All samples were collected aseptically and immediately transported to the laboratory in insulated ice boxes to maintain an appropriate temperature and prevent microbial multiplication during transportation [26]

2.2. Preparation of Samples

Under sterile conditions, 25 g of each sample was aliquoted into sterile bags containing 225 mL of sterile 0.1% peptone water, as previously described [27]. The mixture was homogenized for 30 s at 230 rpm and incubated at room temperature for 5 min. Serial decimal dilutions were prepared by transferring 1 mL of homogenized solution to 9 mL of sterile peptone water.

2.3. Determination of Total Aerobic Bacterial Count (APC)

The total aerobic bacterial count was determined following the Yemeni Standard Specification [28]. Appropriate dilutions (10^{-2}, 10^{-3}, and 10^{-4}) of homogenized samples were prepared. One milliliter of each dilution was transferred to sterile Petri dishes (90 mm diameter). Approximately 15–20 mL of molten nutrient agar (cooled to 45 ℃) was poured into each plate, carefully mixed, and allowed to solidify, invert, and incubated at 30 ± 1 ℃ for 72 h. Colony-forming units (CFU) were counted and the bacterial load was expressed as CFU/g of the sample [29].

2.4. Enumeration of *Pseudomonas* spp

Enumeration of *Pseudomonas* spp. was conducted according to ISO 13720:2010 standards [11]. One milliliter of the homogenized sample was inoculated onto sterile Petri dishes (90 mm diameter) containing

cephalothin–fucidin–cetrimide (CFC) agar supplemented with glycerol (HiMedia Laboratory Ltd., India) at 15–20 mL per plate. The plates were then incubated at 25 ℃ for 48 h. Colonies with greenish-yellow pigmentation were identified as *Pseudomonas* spp.

2.5. Identification of *Pseudomonas* Species

Suspected colonies were subcultured on nutrient agar (HiMedia Laboratory Ltd., India) and incubated at 37 ℃ for 24 h. Purified isolates were subjected to morphological and biochemical characterization. The automated VITEK® 2 COMPACT (bioMérieux, Marcy-l'Étoile, France) is an automated microbial identification system that was developed as a result of bioMérieux's long-standing experience with microbial identification, including oxidase, catalase, and growth characteristics, in accordance with [11, 29].

2.6. Data analysis:

Descriptive statistics including means, standard deviations, and percentages were used to summarize the data. No inferential statistical analyses (e.g., hypothesis testing or confidence intervals) were performed, as the study was primarily designed for surveillance and baseline prevalence estimation rather than for comparative or causal inference. This approach was deemed appropriate, given the absence of group comparisons and the study's focus on assessing microbiological quality in a cross-sectional manner.

3. RESULTS AND DISCUSSION

3.1. Distribution of Samples

As shown in [Table 1], the collected samples comprised a variety of imported meat products that are commonly available in the Yemeni retail market. Chicken meat accounted for the highest proportion (38.32%), followed by minced beef (20.56%) and beef burgers (17.29%).

This distribution likely reflects the prevailing consumer preferences in Yemen, where chickens are considered more accessible and cost-effective than red meat. The absence of mixed-product categories such as "chicken and lamb" may indicate either low market availability or limited consumer demand for such items. The inclusion of diverse product types ensures the representativeness of the sample set and provides a reliable foundation for assessing the microbiological quality of commonly consumed meat.

The distribution also reflects the composition of the imported meat sector in Yemen, where refrigeration infrastructure is often inconsistent owing to frequent power outages and logistical constraints. Thus, analyzing a representative variety of meat offers valuable insights into contamination risks across different product forms, especially given their differing levels of processing and exposure.

3.2. Aerobic Plate Count (APC) and Microbial Load

The aerobic plate count (APC) results, summarized in [Table 2], revealed significant differences in microbial load between product types. Minced beef and minced lamb showed the highest mean counts, at 8.87 × 10^5 CFU/g and 8.55 × 10^5 CFU/g, respectively. These values were markedly higher than those recorded for beef burgers, chicken burgers, and chicken meat, each of which averaged below 1.5 × 10^2 CFU/g.

The elevated microbial load in minced meats can be attributed to their increased surface area and the more extensive handling they undergo during processing, both of which facilitate microbial colonization. Similar trends were reported by Abdelrahman et al. [30] and Edris et al. [31] for comparable meat products. Furthermore, previous studies have noted that mechanical disruption of muscle tissues during mincing exposes the interior to potential contaminants, thereby increasing the susceptibility of the product to microbial growth [3, 32].

Notably, although most burger and chicken samples remained within the acceptable APC thresholds, a few exceeded the expected limits, indicating variability in production hygiene. This underscores the importance of strengthening hygiene practices during the grinding, packaging, and storage stages, especially for minced products that have the highest microbial burden.

3.3. Detection of *Pseudomonas* spp.

Pseudomonas spp. were detected in 16.36% of the analyzed samples, while 83.64% tested negative, as illustrated in Figure 1. Despite the majority of samples complying with general microbiological limits, the detection of spoilage-associated *Pseudomonas* organisms in over one-sixth of samples is microbiologically significant, particularly given their known ability to proliferate under chilled aerobic storage conditions. This finding is consistent with that of an earlier study by Wickramasinghe et al. [33], who emphasized the economic and quality impact of *Pseudomonas psychrophilus* in refrigerated meat. *Pseudomonas* is known for its ability to survive harsh environmental conditions, suppress the growth of other microbes, and cause rapid sensory deterioration. The presence of *P. psychrophilus* in meat, even at low levels, can significantly reduce shelf life and pose a significant risk to product acceptance [15, 16], These

Table[1]: Sample Description and Distribution

Meat Product Type	Meat Source	Number	Percentage (%)
Burger	Beef	37	17.29
Burger	Chicken	34	15.89
Chicken	Chicken	82	38.32
Minced	Beef	44	20.56
Minced	Lamb	17	7.94
Total	—	214	100.00

spoilage effects are largely driven by the secretion of lipases and proteases, which hydrolyze fats and proteins in meat, producing rancid odors, slime formation, and texture degradation [33]. Therefore, it is recommended that stringent monitoring protocols and rapid screening tests be implemented for high-risk meat products, such as minced beef and lamb, to mitigate enzyme-mediated spoilage and maintain product quality.

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The use of traditional culture-based methods for detection is appropriate given the available laboratory infrastructure in the region. However, molecular methods (e.g. as PCR and 16S rRNA sequencing) can provide faster and more precise identification. Although not required by the current ISO 13720:2010 specification, the inclusion of molecular diagnostics in future surveillance efforts could enhance specificity and support early spoilage prediction, particularly for high-risk products, such as minced meat.

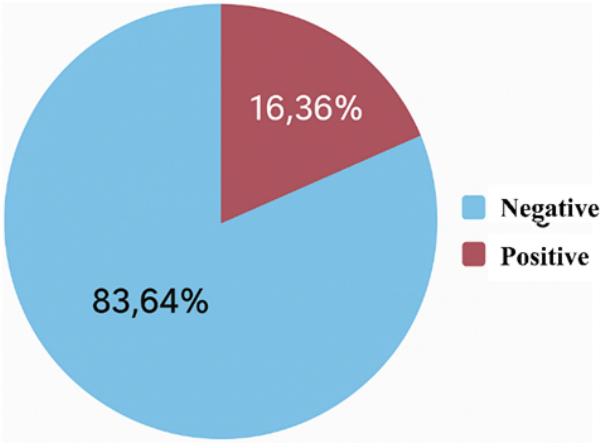

Figure 1. Presence of *Pseudomonas* spp. in Samples

3.4. Comparison with National and International Standards

The compliance of the meat samples with national and international microbiological standards is summarized in [Table 3]. Under the Yemeni Standard (YSMO GSO 1016:2019) [23], all 214 samples (100%) were considered compliant, because this standard does not require testing for P*seudomonas* spp. However, according to the ISO 13720:2010 standard [11], which mandates the absence of *Pseudomonas* spp. in chilled meat products, only 83.64% of samples met the acceptance criteria. The remaining 16.36%, which tested positive for *Pseudomonas* spp., were considered non-compliant.

Table[2]: Average Total Count (APC) by Meat Product Type and Source

Meat Product Type	Meat Source	Mean APC (CFU/g)	Max APC (CFU/g)	Min APC (CFU/g)
Burger	Beef	1.86×10^4	3.55×10^5	1.10×10^2
Burger	Chicken	1.01×10^4	9.70×10^4	1.40×10^2
Chicken meat	Chicken	4.98×10^4	8.60×10^5	1.10×10^2
Minced	Beef	8.87×10^5	5.70×10^6	5.30×10^3
Minced	Lamb	8.55×10^5	6.90×10^6	1.90×10^3

Table[3]: Comparison of *Pseudomonas* spp. Results with Gulf and ISO Standards

Parameter	Gulf Standard (YSMO GSO 1016:2019)	ISO Standard (ISO 13720:2010)	Positive Samples (n)	Negative Samples (n)	% Meeting Gulf Standard	% Meeting ISO Standard
Pseudomonas spp.	Not required	Required	35	179	100.00%	83.64%

For the statistical analysis, only mean values and percentages were calculated and reported. Inferential statistics (e.g., ANOVA or Chi-square) were not applied because of the descriptive and screening-oriented nature of the study, where the primary objective was to assess overall compliance and prevalence patterns rather than to test specific hypotheses. Given its limited resources and scope, this approach was considered appropriate for meeting the study's goals of surveillance and baseline assessment.

This disparity highlights a critical gap between national and international quality assurance frameworks. Although the national standard reflects the current regulatory capacity of Yemen, the omission of specific spoilage organisms, particularly those such as *Pseudomonas* spp., limits its utility in assessing meat freshness and shelf-life risk. These results align with the position of ISO 13720:2010, which emphasizes the role of *Pseudomonas* spp. as an important indicator of spoilage under refrigeration. Given the widespread challenges in cold chain management in Yemen, including power outages and limited regulatory enforcement, the adoption of internationally harmonized microbiological criteria would provide a more accurate reflection of product safety. Updating national standards to align with ISO requirements would enhance meat safety, inform import regulations, and reduce spoilage-related economic loss.

3.5. Acceptance Based on *Pseudomonas* spp. Presence

The acceptability of meat samples based solely on *Pseudomonas* spp. detection is shown in [Table 4]. As expected, all samples were deemed acceptable under the YSMO standard. However, under ISO standards, acceptance rates vary significantly according to the product type. Chicken burgers showed 100% compliance, whereas minced lamb and beef had the lowest acceptance rates of 64.71% and 75.00%, respectively.

These findings reaffirm the earlier observation that minced products are particularly prone to microbial contamination. This is likely due to the combination of mechanical grinding, larger surface area, and greater exposure to potential post-processing contamination. As noted in [35], minced and processed meats tend to be less microbiologically stable and require more rigorous hygiene and monitoring procedures. The lower acceptance rates among minced products, particularly under the ISO criteria, highlight the importance of revising national policies to incorporate spoilage organism testing, particularly for higher-risk items. Enhancing sanitation at processing plants, enforcing better packaging protocols, and ensuring cold chain reliability are vital for minimizing the microbial risks in these categories.

3.6. Species Distribution of *Pseudomonas* spp. Isolates

Species-level identification of *Pseudomonas* isolates, as detailed in [Table 5], showed that *Pseudomonas aeruginosa* accounted for 60% of all identified isolates, followed by *Pseudomonas fluorescens* at 40%. *P. aeruginosa* was most frequently recovered from chicken and minced meat samples, whereas *P. fluorescens* was more common in burger products.

The predominance of *P. aeruginosa* is of particular concern because of its recognized spoilage potential and public health relevance. This opportunistic pathogen is capable of biofilm formation, environmental persistence, and resistance to various sanitizers. The frequent isolation in this study reinforces the findings of Khalafallah et al. [36], who identified *P. aeruginosa* as the dominant species in frozen minced meat samples. Farghaly et al. [37], reported the notable presence of *Pseudomonas* species, including *P. aeruginosa* in both burgers and minced meat.

Although *P. fluorescens* is primarily associated with spoilage rather than infection, its psychrotrophic properties make it a reliable indicator of cold-storage deterioration. As recommended by ISO 13720:2010, species-level monitoring improves the accuracy of microbial quality assessments and allows for more targeted control measures in food safety management systems.

These results demonstrate the added value of identifying *Pseudomonas* species at the genus and species levels as different strains vary in their spoilage dynamics, pathogenic potential, and resistance profiles. Although this study utilized conventional and automated biochemical identification methods, molecular confirmation would further strengthen species-level accuracy.

Table[4]: *Pseudomonas* spp. Acceptance by Meat Product Type and Source

Meat Product Type	Meat Source	YSMO GSO 1016:2019 Acceptance (%)	ISO 13720:2010 Acceptance (%)
Burger	Beef	100.00%	89.19%
Burger	Chicken	100.00%	100.00%
Chicken meat	Chicken	100.00%	82.93%
Minced	Beef	100.00%	75.00%
Minced	Lamb	100.00%	64.71%

In addition to traditional microbiological inquiries, significant challenges persist in microbial detection and spoilage-organism monitoring in low-resource settings, such as Yemen. Many laboratories struggle to operate at optimal levels, owing to the lack of rapid, sensitive, and cost-effective diagnostic methods. As highlighted by Al-Nowihi et al. [38], discrepancies may sometimes arise between conventional techniques (e.g., agglutination tests) and more advanced tools (e.g., enzyme-linked immunosorbent assays). This underscores the urgent need to enhance laboratory infrastructure and adopt standardized testing platforms to ensure the accurate and reliable identification of microbial hazards. Surveillance of spoilage organisms, such as *Pseudomonas* spp., faces similar obstacles, and integrated diagnostic strategies that combine traditional and molecular approaches are potentially more effective.

These diagnostic and infrastructure gaps are not limited to the food sector. Broader health-related microbiological issues in Yemen reveal a consistent pattern of under-diagnosis, limited awareness in human populations, and a high prevalence of infection in animal populations. Multiple studies have highlighted the elevated exposure rates to infectious agents in the general population, especially among vulnerable groups. For example, Al-Arnoot et al. [39] documented widespread challenges in managing zoonotic risks under constrained laboratory capacity. Similarly, studies focusing on women's health have reported inconsistent screening practices and low awareness levels [40, 41]. Furthermore, in patients with chronic conditions, diagnostic gaps have been shown to exacerbate complications, with co-infections often going undetected owing to insufficient diagnostic systems [42].

Cumulative evidence from these and other studies points to the need for national-level surveillance systems and targeted awareness initiatives. There is also an urgent demand for operational research to address local knowledge gaps and support the implementation of practical and cost-effective diagnostic tools suitable for both healthcare and field use. Enhancing diagnostic capabilities would not only strengthen the health sector, but also improve microbial monitoring across food supply chains.

Furthermore, the integration of natural preservatives is a promising avenue for enhancing food safety and combating microbial spoilage. *Boswellia sacra*, a resinous plant native to the South Arabian Peninsula, has demonstrated broad antimicrobial potential and effectively reduces the growth of various spoilage and pathogenic organisms. According to the findings reviewed by Abdullah et al. [43], this plant extract holds promise for applications in food preservation systems. Further research is warranted to evaluate its effectiveness, safety, and sensory impact when

Table[5]: Types of Isolated *Pseudomonas* spp.

Meat Product Type	Meat Source	*Pseudomonas* Species	Number	Percentage (%)
Burger	Beef	*P. aeruginosa*	3	8.57%
Burger	Beef	*P. fluorescens*	1	2.86%
Chicken meat	Chicken	*P. aeruginosa*	8	22.86%
Chicken meat	Chicken	*P. fluorescens*	6	17.14%
Minced	Beef	*P. aeruginosa*	6	17.14%
Minced	Beef	*P. fluorescens*	5	14.29%
Minced	Lamb	*P. aeruginosa*	4	11.43%
Minced	Lamb	*P. fluorescens*	2	5.71%
Total	—	*P. aeruginosa*	21	60.00%
		P. fluorescens	14	40.00%

incorporated into meat packaging or formulation materials as part of a comprehensive approach to shelf-life extension and spoilage prevention.

Limitations

This study was designed as a packaged microbiological quality assessment based on standard culture and biochemical analyses. Although these culture-based methods remain widely used and are required by ISO 13720:2010, they have inherent limitations in detection speed and resolution. Molecular methods, such as PCR and 16S rRNA gene sequencing, are faster and more precise for species-level identification and can detect non-culturable or slow-growing spoilage organisms. These techniques were not applied in the current study because of resource constraints; however, their integration in future research is strongly recommended to enhance the diagnostic accuracy and credibility. In the present study, the VITEK 2 Compact system was employed for species-level identification, offering high reliability, although occasional misidentifications may occur in closely related strains if molecular confirmation is not performed.

In addition, the analysis relied exclusively on descriptive statistics (mean values and percentage occurrence rates). This approach was deemed appropriate given the study's primary aim of evaluating prevalence and comparing results against standard thresholds, rather than testing specific hypotheses. Nevertheless, the observed contamination patterns between product types provide valuable insights into microbial risks in Yemen's meat supply chain, and future studies incorporating inferential statistics could strengthen comparative analyses.

4. CONCLUSION

This study demonstrated that imported minced meat products in Yemen, particularly minced beef and lamb, exhibited higher microbial loads and a greater prevalence of *Pseudomonas* spp., including spoilage-associated *P. aeruginosa.* Although most samples met the national standards, non-compliance with ISO 13720:2010 highlights the need to strengthen microbiological monitoring frameworks. Incorporating *Pseudomonas* spp. testing, reinforcing hygiene, and cold chain management are essential steps toward improving meat safety and preserving product quality in the Yemeni market.

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