Original Article
IASR Journal of Medical and Pharmaceutical Science
Website: https://iasrpublication.com/ijmps/journal
ISSN (online): 2582-9343
Page No : 01-06

Microbiological identification of *Pantoea spp.* bacteria from pet food products

Dhulfiqar Abbas Aswad[1] & Sabaa Hilal Hadi[1*]

[1]Department of Microbiology, College of Veterinary Medicine, Al-Qasim green University, Iraq.

ABSTRACT

Humans, especially children, are in constant contact with pets and therefore with everything related to them, such as food, so we took 60 samples of cooked and dried pet food for laboratory examination and to investigate whether it contains pathogenic bacteria for humans. The samples were taken from the Companies (Jungle, Hellaw, Pado, Vitus, LoLo, Paw, Pedigree, Dr. Clauders) in Iraq. The results revealed that the isolated *Pantoea spp.* exhibited characteristic features on the different media. On XLD Agar, the colonies appeared yellow; on MacConkey Agar, the colonies were pink, and on Blood Agar, they exhibited beta-hemolysis. Microscopically, the bacteria appeared as short, Gram-negative, rod-shaped cells arranged singly, The study also examined the growth of samples on different culture media, with 100% growth observed on Blood Agar, 28.3% on MacConkey Agar, 30.0% on XLD Agar, and 20.0% on a mixed media of MacConkey and XLD Agar. The Vitek2 system was used for the conclusive identification of 60 isolates of *Pantoea spp.*, with accuracy ranging from 95% to 99%. The total count determination showed bacterial loads ranging from 9.2 x 10^6 to 2.7 x 10^7 log CFU per milliliter in pet nutrient products.

Keywords*: Pantoea spp., pet food, Vitek2 system*

Corresponding Author
Sabaa Hilal Hadi
Department of Microbiology, College of Veterinary Medicine, Al-Qasim green University, Iraq.

INTRODUCTION

Microorganisms found in pet food pose a dual health hazard, affecting not only the pets themselves but also their owners who share close bonds with them. Studies have demonstrated that contaminated pet food can lead to human illnesses through various means, including direct handling of the food and indirect exposure through contact with objects that have been in contact with pet food. Additionally, certain pets may carry these diseases without showing any symptoms [1].

The pet population in Europe is steadily rising, with approximately 80 million households owning at least one pet animal [2]. Pets hold a significant place in the lives of those who consider them as "family members" [3,4,5]. This increasing trend in pet ownership has led to a dynamic development of the pet food market. Pet food has become widely popular among animal owners due to its convenience, cost-effectiveness, and easy availability throughout their pets' lives. The pet food industry is experiencing an annual growth rate of approximately 2.6% [2].

Currently, there is a growing trend of pet ownership, particularly of cats and dogs, worldwide. Reports indicate that approximately 80 million European households [6] and 60% of households in the United States [7] own at least one pet.This increase in pet ownership has been further highlighted during the COVID-19 pandemic, as pets have been recognized as companions that provide comfort and contribute to individuals' well-being [8]. As the number of pets continues to rise, the pet food market is also experiencing significant growth. The manufacturing of pet food has been established in Europe and the United States since the 1940s, initially utilizing animal feed intended for livestock. Nowadays, most developed countries have dedicated pet food manufacturing facilities, reflecting the evolving nature of the industry [9].

The genus *Pantoea*comprises 20 distinct species, which are *named Pantoeaeucalyptii, Pantoeaagglomerans, Pantoeavagans, Pantoeaconspicua, Pantoeadeleyi, Pantoeaanthophila, Pantoeabrenneri, Pantoeaananatis, Pantoeaallii, Pantoeastewartii, Pantoeacypripedii, Pantoeacalida, Pantoeagavinae, Pantoeadispersa, Pantoeaseptica, Pantoeawallisii, Pantoeaeucrina, Pantoearodasii, Pantoearwandensis, and Pectobacteriumcarotovorum.* The representative strain chosen for this genus is *Pantoeaagglomerans*[10].

The study aimed to isolate bacteria in pet food products and identify bacteria using Vitek 2 Compact.

Collection of specimens

60 samples were taken from two types of pet meals (30 Cooked and 30 Dried). A representative sample is collected from the pet meal using a sterile utensil or spoon during the period from October to December 2022 in the laboratory of the College of Veterinary Medicine at Al-qasim Green University. The samples were collected from the Companies (Jungle, Hellaw, Pado, Vitus, LoLo, Paw, Pedigree, Dr. Clauders) in Iraq. Upon arrival at the laboratory, the sample is homogenized to ensure an even distribution of the bacteria in the specimen. This can be done using a sterile blender or by vigorously shaking the container.

The isolation of bacteria was done based on the general principles of culturing by using Blood, XLD, and MaCconkey agar by incubation at 37°C for 24 h to study their morphological characteristics and microscopic examination with Gram stain.

Total bacterial count determination

According to Sanders, [11],determining the total bacterial count is a straightforward method for obtaining manageable concentrations of a specific organism. This technique involves streaking and spreading the sample on a nutrient-rich agar medium in petri dishes. The plates are then incubated at 37°C for 16-24 hours, and the resulting colonies on each plate are counted. The acceptable range for counting is typically between 30 and 300 colony-forming units (CFUs) per plate. The calculation of CFU/mL is performed using the formula: CFU/mL = number of counted colonies * 10 * 10^(dilution factor).

Identification of bacteria by Vitek 2 system

The biochemical identification of the isolated *Pentoea spp.* was completed by the VITEK 2 compact system, which depends on 62 biochemical and physiological test responses. The VITEK 2 compact system is a completely computerized process that provides bacterial detection via biochemical examination using colorimetry. This system is doing all the processes that are needed for this microbe identification and permits dynamic examination by perusing every test in 15 minutes [12].

Statistical Analysis

Statistical analysis of the results by using a computer program (SPSS), Version 28. The data were shown in simple measures of frequency, and percentage. The significance of the difference for different percentages (qualitative data) was tested using the Pearson Chi-square test (2-test). Statistical significance was taken into account when the P-value was equal to or less than 0.05.

Results and Discussion

Identification of the bacteria:

The identification of the bacteria was ensured according to the (standard operating procedures (2007) by following these steps:

Cultural characteristics

Pantoea spp. are typically Gram-negative bacteria that are facultative anaerobes, meaning they can grow both in the presence and absence of oxygen. They are often motile, possessing flagella that allow them to move in liquid environments.

Table (1) presents the morphology, cultural characteristics, and staining characteristics of isolated *Pantoea spp.* The isolates were cultured on different media, including Xylose-Lysine Deoxycholate (XLD) Agar, MacConkey Agar, and Blood Agar. On Xylose-Lysine Deoxycholate (XLD) Agar, the colonies appeared yellow. When grown on MacConkey Agar, the colonies displayed a pink color. On Blood Agar, the colonies exhibited beta-hemolysis, indicating the destruction of red blood cells. Microscopically, the Pantoea spp. isolates showed a Gram-negative staining pattern, appearing as short rod-shaped cells that were arranged singly. These findings provide valuable information for the preliminary identification and characterization of *Pantoea spp.*

Pantoea spp. are known to be nutritionally versatile and can utilize a wide range of carbon sources for growth. This versatility allows them to thrive in various ecological niches, including soil, plants, water, and even as opportunistic pathogens in humans and animals [10].

These factors included the observation of the swarming phenomenon on blood agar, the distinct smell exhibited by the cultures, and the pale appearance of bacteria (non-lactose fermenting) on MacConkey agar. Furthermore, the identification involved the examination of convex, round, and smooth colonies that emit a characteristic fishy odor [13].

MacConkey agar is utilized for primary identification due to its inclusion of bile salts and crystal violet, which promote the growth of Enterobacteriaceae and associated intestinal Gram-negative rods. Simultaneously, it inhibits the growth of Gram-positive bacteria and certain fastidious Gram-negative bacteria. The presence of lactose as the sole carbon source in this medium enables differentiation between lactose-fermenting and non-lactose-fermenting bacteria.

However, it is worth noting that the colonies of non-lactose-fermenting bacteria may appear pale or translucent, which is considered unfavorable [14].

Table (1): Morphology, cultural, and staining characteristics of isolated Pantoea spp.

Media Used	Colony Characteristics	Morphology (Staining Characteristics)
Xylose-Lysine Deoxycholate (XLD) Agar	Yellow colonies	Gram-negative, short rod-shaped, singly or paired short arranged Appearance
MacConkey Agar	Pink colonies	
Blood Agar	Beta-hemolytic colonies	

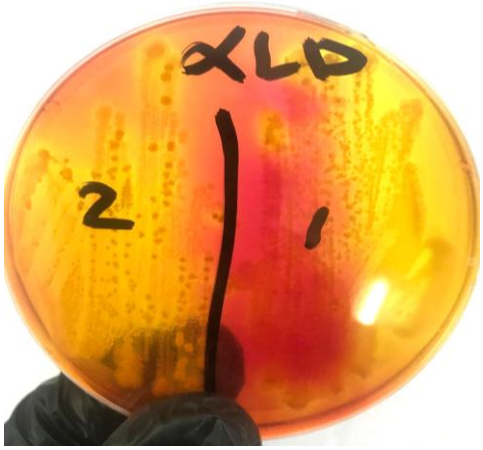

Figure (1): Pantoea yellow colonies XLD agar acidify the medium, turning it yellow

Figure (2): Pantoea pink colonies MacConkey Agar

Table 1 presents the distribution of the studied samples according to the culture media used for bacterial growth assessment. A total of 60 samples were analyzed using different culture media, including Blood Agar, MacConkey Agar, Xylose-Lysine Deoxycholate (XLD) Agar, and a mixed media of MacConkey Agar and XLD Agar. The results indicate that all 60 samples (100.0%) showed growth on Blood Agar, indicating the presence of bacteria that can grow on this medium. MacConkey Agar and XLD Agar also supported bacterial growth, with 17 samples (28.3%) showing growth on MacConkey Agar and 18 samples (30.0%) showing growth on XLD Agar. Interestingly, a subset of samples (12 samples, 20.0%) exhibited growth on the mixed media of MacConkey Agar and XLD Agar, suggesting that this combination allowed for the detection of bacterial species that may not have grown on the individual media alone. The distribution of growth among the different culture media indicates the presence of diverse bacterial populations within the studied samples. Different media support the growth of specific groups of bacteria based on their nutritional requirements and metabolic characteristics. The use of multiple culture media in this study enhances the ability to detect a broader range of bacterial species and provides a more comprehensive assessment of the microbial composition present in the samples. It is important to note that the growth observed on these media does not necessarily indicate the presence of pathogenic bacteria but rather represents the overall bacterial load and diversity. The results from this distribution analysis can serve

as a foundation for further investigations, such as the identification and characterization of specific bacterial species within the samples. Additionally, the information obtained from this study can contribute to understanding the microbial ecology and potential health implications associated with the studied samples.

Table (2) Distribution of the studied samples according to culture medium

	Blood Agar	MaCconky Agar	XLD	Mixed (MaCconky + XLD)
Total number of samples = 60	Growth No. (%)	Growth No. (%)	Growth No. (%)	Growth No. (%)
	60 (100.0%)	17 (28.3%)	18 (30.0%)	12 (20.0%)

Table 3 presents the distribution of the studied samples based on the type of nutrients (pet animals) and the growth observed on MacConkey Agar and Xylose-Lysine Deoxycholate (XLD) Agar. The Chi-square (X2) test was used to assess the association between the variables, and the p-value was calculated to determine the statistical significance of any observed associations. The table shows that out of the total samples analyzed, 82.4% of the cooked nutrient samples showed growth on MacConkey Agar, while 94.4% showed growth on XLD Agar. For the dried nutrient samples, 17.6% exhibited growth on MacConkey Agar, and 5.6% showed growth on XLD Agar. When considering the overall distribution, 88.6% of the total samples of cooked nutrients showed growth on MacConkey Agar, compared to 11.4% of the dried nutrient samples. Similarly, 94.4% of the cooked nutrient samples showed growth on XLD Agar, while only 5.6% of the dried nutrient samples exhibited growth on this medium. These findings indicate that the type of nutrients (cooked or dried) does not have a significant impact on the growth of bacteria on the MacConkey Agar and XLD Agar.

Table (3) Distribution of the studied samples according to the type of nutrient

			MacConkey Agar	XLD	Total	X2	P. value
Type of nutrients (pet animal)	Cooked	No.	14	17	31	1.263	0.261
		%	82.4%	94.4%	88.6%		
	Dried	No.	3	1	4		
		%	17.6%	5.6%	11.4%		

Pearson Chi-Square (X2); Significant <0.05

To the best of our knowledge, there is no study that has dealt with the presence of *pantoea* bacteria in pet food, but the current study can be compared to previous studies that examined the presence of other types of bacteria in pet food. In 2018, the Centers for Disease Control and Prevention (CDC) investigated a multistate outbreak of Salmonella infections linked to raw pet food. The investigation revealed that individuals became ill after handling contaminated raw pet food products. The CDC emphasized the importance of safe handling practices when dealing with raw pet food [15].

In a study conducted by Hellgren *et al*., [16] on raw meat-based diets (RMBDs) for dogs, frozen samples from 60 RMBD packs produced by 10 different manufacturers were examined for the presence of various bacteria. The researchers focused on bacteria belonging to the family Enterobacteriaceae, Clostridium perfringens, as well as Salmonella and Campylobacter. The results indicated that Enterobacteriaceae were present in all 60 samples, with 31 samples surpassing the threshold of 5000 bacteria/g, which is considered satisfactory microbial hygiene as per EU regulations. Two samples exceeded the limit of 5000 bacteria/g for C. perfringens, the maximum level of anaerobic bacteria permitted according to Swedish national guidelines. Salmonella species were detected in four samples (7 percent), and Campylobacter species were found in three samples (5 percent).

Vitek2 system identification of Pantoea

Using the Vitek2 technique, which correctly recognized all isolates as *Pantoea* with a 95% accuracy rate, the diagnosis of *Pantoea*was finally established. Table (4) contains the full findings of this investigation.The Vitek2 system is a suitable device for rapid and direct diagnosis of bacterial isolates at the species level [17].

The Vitek 2 system employs a combination of biochemical reactions, growth patterns, and metabolic activities to identify *Pantoea* species. The system analyzes the response of the bacterial isolate to various substrates and generates a profile that is compared to a comprehensive database to determine the most probable identification [18].

Table (4): Identification information of *Pantoea spp.* By Vitek 2 compact system

Identification Information	**Analysis Time:**	7.78 hours	**Status:**	Final
Selected Organism	95% Probability **Bionumber:**	**Pantoea spp** 4607710143540210		
ID Analysis Messages				

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Total bacterial count determination

The current study found that the total bacterial count determination was performed to assess the bacterial load of Pantoea spp. in pet nutrient products, specifically in dried and cooked forms. The results indicated a range of total bacterial counts from 9.2 x 10^6 to 2.7 x 10^7 log colony-forming units (CFU) per milliliter (ml). This logarithmic scale reflects the number of viable bacterial cells present in the sample. This study agreed with a study conducted by Kazimierska et al., [19], where the total aerobic microbial count in the examined dry dog foods ranged from 2.7 × 10^2 to above 3.0 × 10^7 colony-forming units per gram (cfu/g).

In a study conducted by Serhan et al., [20], it was found that dry pet food products exhibited a higher level of contamination compared to canned products. Regarding conformity to European Commission regulations, out of the 165 brands analyzed, 11 (7%) had a total aerobic microbial count exceeding 106 cfu/g, and 27 (16%) surpassed the maximum limit of presumptive Enterobacteriaceae, which is 3 × 10^2cfu/g. Specifically focusing on dry pet food brands, 8 out of 66 (12%) showed a contamination level of yeasts and molds above 10^4cfu/g. These findings suggest that there is a notable proportion of pet food brands that do not meet the required microbial quality standards, particularly in terms of total aerobic microbial count.

Microscopic examination

Pantoea spp. typically appear as Gram-negative, short, rod-shaped bacteria. They are often observed as individual cells or arranged in pairs or short chains. The cells are usually straight or slightly curved. *Pantoea spp.* stain negatively during the Gram staining procedure, indicating that they do not retain the crystal violet dye and appear pink or red after counterstaining with safranin. This indicates their Gram-negative nature. As explained in Figure 3.

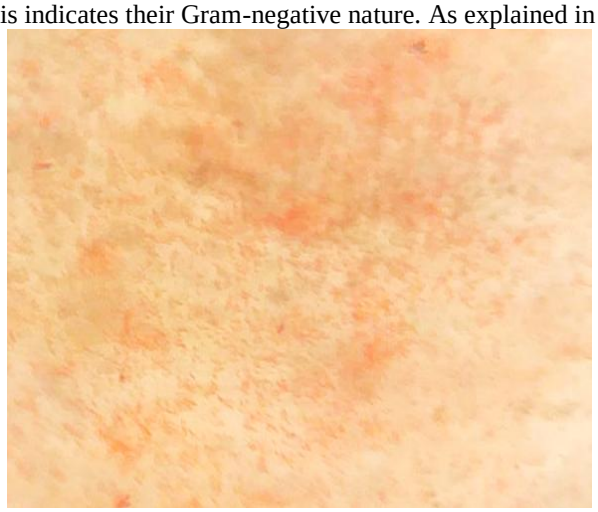

Figure (3): Microscopic examination of *Pantoea spp.*

REFERENCES

1. Center for Veterinary Medicine. (2022). *Get the Facts about Listeria. U.S. Food and Drug Administration*. https://www.fda.gov/animalveterinary/animal-health-literacy/get-facts-about-listeria
2. FEDIAF. (2020b). *the FEDIAF Nutritional Guidelines for Complete and Complementary Pet Food for Cats and Dogs*. https://europeanpetfood.org/wp-content/uploads/2022/03/Updated-Nutritional-Guidelines.pdf
3. Di Cerbo, A., Morales-Medina, J. C., Palmieri, B., Pezzuto, F., Cocco, R., Flores, G., & Iannitti, T. (2017). Functional foods in pet nutrition: Focus on dogs and cats. *Research in Veterinary Science*, *112*, 161–166.
4. Rauktis, M. E., Rose, L., Chen, Q., Martone, R., & Martello, A. (2017). "Their pets are loved members of their family": Animal ownership, food insecurity, and the value of having pet food available in food banks. *Anthrozoös*, *30*(4), 581–593.
5. McConnell, A. R., Paige Lloyd, E., & Humphrey, B. T. (2019). We are family: Viewing pets as family members improves wellbeing. *Anthrozoös*, *32*(4), 459–470.
6. FEDIAF. (2020a). *Annual Report. The European Pet Food Industry, Bruxelles*.
7. Applebaum, J. W., Peek, C. W., & Zsembik, B. A. (2023). Examining US pet ownership using the General Social Survey. *The Social Science Journal*, *60*(1), 110–119.
8. Hoy-Gerlach, J., Rauktis, M., & Newhill, C. (2020). (Non-human) animal companionship: A crucial support for people during the COVID-19 pandemic. *Society Register*, *4*(2), 109–120.
9. Guy, R. C. E. (2004). *Pet foods. In: Encyclopedia of Grain Science. Australia: Elsevier Academic Press*. 445–450. doi: 10.1016/B0-12-765490-9/00127-0
10. Walterson, A. M., & Stavrinides, J. (2015). Pantoea: insights into a highly versatile and diverse genus within the Enterobacteriaceae. *FEMS Microbiology Reviews*, *39*(6), 968–984
11. Sanders, E. R. (2012). Aseptic laboratory techniques: plating methods. *JoVE (Journal of Visualized Experiments)*, *63*, e3064.
12. Ligozzi, M., Bernini, C., Bonora, M. G., De Fatima, M., Zuliani, J., & Fontana, R. (2002). Evaluation of the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant gram-positive cocci. *Journal of Clinical Microbiology*, *40*(5), 1681–1686.
13. Forbes, B. A., Sahm, D. F., & Weissfeld, A. S. (2007). Bailey & Scott's Diagnostic Microbiology. Mosby Elsevier; St. *Louis, MO*.
14. Osman, E. A., El-Amin, N., Adrees, E. A. E., Al-Hassan, L., & Mukhtar, M. (2020). Comparing conventional, biochemical and genotypic methods for accurate identification of Klebsiella pneumoniae in Sudan. *Access Microbiology*, *2*(3).
15. Hassan, R., Buuck, S., Noveroske, D., Medus, C., Sorenson, A., Laurent, J., Rotstein, D., Schlater, L., Freiman, J., & Douris, A. (2019). Multistate outbreak of Salmonella infections linked to raw turkey products—United States, 2017–2019. *Morbidity and Mortality Weekly Report*, *68*(46), 1045.
16. Hellgren, J., Hästö, L. S., Wikström, C., Fernström, L., & Hansson, I. (2019). Occurrence of Salmonella, Campylobacter, Clostridium and Enterobacteriaceae in raw meat-based diets for dogs. *Veterinary Record*, *184*(14), 442.
17. Nimer, N. A., Al-Saa'da, R. J., & Abuelaish, O. (2016). Accuracy of the VITEK 2 system for a rapid and direct identification and susceptibility testing of gram-negative rods and gram-positive cocci in blood samples. *EMHJ-Eastern Mediterranean Health Journal*, *22*(3), 193–200.
18. Jorgensen, J. H., & Turnidge, J. D. (2015). Susceptibility test methods: dilution and disk diffusion methods. *Manual of Clinical Microbiology*, 1253–1273.
19. Kazimierska, K., Biel, W., Witkowicz, R., Karakulska, J., & Stachurska, X. (2021). Evaluation of nutritional value and microbiological safety in commercial dog food. *Veterinary Research Communications*, *45*(2–3), 111–128.
20. Serhan, M., Hadid, M., Dimassi, H., Deghel, M., & Hassan, H. F. (2022). Microbiological safety of commercial canned and dry pet food products in Lebanon. *Frontiers in Veterinary Science*, *9*, 995184.