DF-602
[1-8]

RESEARCH ARTICLE Agricultural Science Digest, Volume Issue : ()

Enterobacter hormaechei Bacteria from Paper Napkins Confirmed by 16S rRNA Gene and Experimental Infection in Rats

Sabaa Hilal Hadi[1], Muna A. Al-Khafaji[1], Rana H. Raheema[2], Abdulazeez A. Hasoony[3] **10.18805/ag.DF-602**

ABSTRACT

Background: With the frequent use of paper napkins for everyday personal use and direct contact with the external openings of our bodies such as the nostril, eyes and even the vagina. With its many types and the lack of necessary sterilization methods in factories, it is likely that it contains germs with the possibility of entering them. The current study aimed isolated *E. hormaechei* to experimentally infected and determine the pathogenicity of *E. hormaechei* in rat newborns and adult rats by two ways of infection.

Methods: Confirmed by this unique research with its content by isolating bacteria *Enterobacter hormaechei* at a large presence rate per 0.2 mg of each napkin. In humans, *Enterobacter hormaechei* normally behaves as an opportunistic pathogenic bacteria. Vitek 2 compact biochemical tests were performed on fifty *E. hormaechei* isolates.

Result: Three strains of *E. hormaechei* that were examined and closely linked to Enterobacter spp. had their 16S rRNA gene sequenced using the Sanger method. The bacteria's closest relatives among Gen Bank sequences were *Enterobacter cloacae* and *Enterobacter hormaechei.* (99-100%). Interstitial pneumonia, acute enteritis, necrosis and inflammation-related cell infiltration in the parenchyma of the uterus and atretic oviduct epithelium are all seen in the histopathological examination of affected rats.

Key words: 16S rRNA, *E. hormaechei*, Paper napkins, Pathogenicity.

INTRODUCTION

Only a small portion of the microorganisms present in the environment of paper-processing technologies (those that create toxins and bad tastes) are detrimental to the operation or the security of the finished goods. Microbes typically enter machinery through the raw ingredients and chemicals used to produce paper. They usually produce biofilms on a variety of surfaces and flourish in the conditions of the paper mill. Circulating process water is a tried-and-true technique for preserving and distributing microbial contamination in mills, claim (Suihko and Skytta, 1997). The bacteria that produce enzymes and toxins could cause issues if they diffuse from the paper into food and skin scrapes (Swartz, 2004; Suominen *et al.,* 1997).

Microbiological quality is an important consideration in risk assessment due to disposal fibers providing a favorable environment for bacteria development when materials are collected, storedand processed (Blanco *et al.,* 1996; Sorelle and Belgard, 1991). Although even though the vast majority of food sellers were aware of safe food handling procedure, they were not properly followed when preparing food, which lead to increased levels of microbial contamination in their produce (Krishnasree *et al.,* 2018).

The species of the *Enterobacter cloacae* complex includes a large variety of bacteria that have been widely seen in numerous circumstances and have the potential to act as human pathogens (Mezzatesta *et al.,* 2012). These microorganisms are members of the Enterobacteriaceae family, which has been linked to the colonization of the human respiratory system and has the potential to promote pathological progression leading to recurrent lung infections (Abbas *et al.,* 2020). They are also regarded as frequent nosocomial infections (Gaston, 1988) capable of causing various diseases, such as bacteremia, septicemia, pneumonia, cystitis, hospital-acquired bacteremiaand injury contagions (Xiong *et al.,* 2008). These creatures, which today belong to the species *Ent. hormaechei, Ent. dissolvens, Ent. cloacae, Ent. kobei* and *Ent. ludwigii* were discovered to contain highly heterogeneous genomes. In clinical instances, *E. hormaechei* emerged as the most

[1]Department of Pathology and Poultry Diseases, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq.
[2]Department of Microbiology, College of Medicine, Wasit University, Iraq.
[3]Division of Graduate Studies, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013.

Corresponding Author: Sabaa Hilal Hadi,Pathology and Poultry Diseases Department, College of Veterinary Medicine, Al-Qasim Green University, Babylon 51013, Iraq.
Email: sabahelal@vet.uoqasim.edu.iq

How to cite this article: Hadi, S.H., Al-khafaji, M.A., Raheema, R.H. and Hasoony, A.A. (2024). *Enterobacter Hormaechei* Bacteria from Paper Napkins Confirmed by 16S rRNA Gene and Experimental Infection in Rats. Agricultural Science Digest. DOI: 10.18805/ag.DF-602.

Submitted: 31-01-2024 **Accepted:** 03-04-2024 **Online:** 18-07-2024

frequently recognized species (Hoffmann and Roggenkamp, 2003; Morand *et al.,* 2009; Boone and Castenholz, 2001).

For reproducible molecular typing approaches for identification, open, trustworthy and cumulative datasets must be available (Paauw *et al.,* 2009; Abhyankar *et al.,* 2022). This information suggests that both animal and human health may be at risk from the possible spread of this strain. High-quality identification data bases used to be rare, but they were sometimes available for gene sequences encoding particular housekeeping genes.

Numerous environmental niches contain *E. hormaechei* (Davin-Regli *et al.,* 1997). It is frequently regarded as a pathogen that causes nosocomial infections (Paauw *et al.,* 2009) and it seldom affects animals outside humans. Animals with respiratory illness complex (Khalifa *et al.,* 2021) also a dead fox with an infection in its uterus (Shan-Shan *et al.,* 2017), diarrheal piglets Lu-Yao *et al.,* (2017), respiratory disease in calves, goats 0 and sheep (Wang *et al.,* 2020), fish pathogen (Nallaiah *et al.,* 2022), combination of respiratory diseases in fauna (Khalifa *et al.,* 2020) have all tested positive for *E. hormaechei* infections. *E. hormaechei* was just recently identified as the pathogen responsible for pathological alterations in sheep in 2022 (Hongfei *et al.,* 2022).

The goal of this investigation was to detect isolates coming from regular paper napkins and to build a database for personal identification. The later commercial database is more reliable, but it is still restricted to a small number of species that are important for medical and food microbiology and environmental isolates like those from the primary material of the paper industry are not present. We first isolated *E. hormaechei* to experimentally infected and determine the pathogenicity of *E. hormaechei* in rat newborns and adult rats by two ways of infection.

MATERIALS AND METHODS

Ethical approval

The ethical approval for the current experiment was obtained from the ethics committee of Veterinary Medicine at Al-Qasim Green University and approved to guide the care and use of laboratory animals (ESCVM, No. 1192022).

Samples and isolation

The experiment was conducted during the period from of September 2022 to February 2023 at the AL-Qasim green university, Bacteriology department laboratory led to the isolation of 50 aerobic bacterial isolates. Samples from recycled paper napkin (Dima)®, were taken from a factories in Hillah , Babil province, Iraq. A 0.2 mg random sample of each napkin was homogenized in a Stomacher for 1 minute after being combined with a Peptone water tube solution for the culture of the bacteria for 12 hours. For 24 hours, MacConkey agar and Nutrient agar were cultured and incubated at 30°C after receiving a trans-loopful of the solution. By serially diluting 10-fold, you can determine the quantity of colony-forming units and their corresponding concentrations. For the molecular lab, the isolates were kept at 20°C in 5% glycerol.

Physiological and morphological characteristics

The 50 *E. hormaechei* isolates came from paper napkins. Biochemical testing was performed using the Vitek 2 Compactand morphological (cell size and shape, pigment production and Gram reaction) studies were conducted through culture.

PCR reactions and analysis of the 16S rDNA sequence

For three isolates of *E. hormaechei,* Primer sets 143 are for general use (5-AGAGTTTGATCATGGCTCAG-3)and DG 74 is for specific use (5-ACGGTTACCTTGTTACGACTT-3) (Weisburg *et al.,* 1991). “30 cycles of initial denaturation at 94°C for two minutes, annealing at 56°C for thirty seconds, extension at 72°C for four minutes and a final extension at 72°C for ten minutes were performed after the initial denaturation at 94°C for five minutes”. The amplified products were treated in a 1X TBE buffer before being electrophoretically stained on a 1.5% agarose gel with RedSafe Nucleic Acid Stain.

According to the primer synthesizer company’s instructions, the primers (which were initially lyophilized) and the amplified fragment were directly sequenced using the Sanger sequencing method (Macrogen, Korea).

Using the G-spin Genomic DNA Kit, total DNA was extracted from the strain that had been isolated after 20 hours at 37°C in LB broth for three isolates. Following the established methods (Martínez *et al.,* 2014), an Opti-DNA marker gene was used in order to run the previously released polymerase chain reaction (PCR) primers (Poirel *et al.,* 2011).

The partial region of 16S rRNA of *E. cloaca* is 353 bpand the consensus sequence was used to search for homologous sequences at Gen Bank.

Table 1: Vitek 2 compact biochemical test for *E. hormaechei.*

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 -		

Experimental infection

In group one, there are eight male baby rats (2 weeks to 28 days old, weighing 21-35 g). Using the intranasal grip, uniformly distribute 10-20 microliters of *E. hormaechei* suspension (2×10^7 mg/ml CFU) into the nose. Allow the inoculum to flow gradually, allowing the rat enough time to breathe in the inoculum (Southam *et al.,* 2002). Eight adult female rats (2 months post-partum, weighing 63-95 g) are given a volume dose of 0.5 ml of bacterial suspension intra vaginally in group two, while the third group is the control group. Following infection, check the rat every 24-72 hours for medical conditions that cause signs including fatigue, frizzy hairand weight loss.

According to (Abbas *et al.,* 2012) the lungs and uterus of the experimental animals were dissected into histopathological sections using the H and E stain.

RESULTS AND DISCUSSION

The group from which all 50 isolates were cultivated for 24 hours at 30°C on MacConkey agar was identified as *Enterobacter hormaechei.* With a high growth rate, the resulting colonies were round, smooth, pinkand had regular edges that were 1-2 mm in diameter, as shown in Fig 1.

Fig 1: *E. hormaechei* in Macconkey agar.

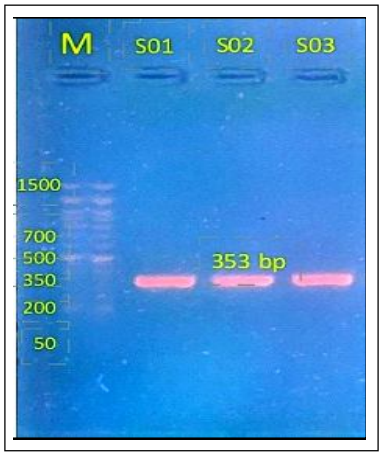

Fig 2: Displays the PCR result relating to the partial 16S rRNA gene of *E. hormaechei* on an agarose gel electrophoresis.

Table 2: Identification of the *E. hormaechei* 16S rRNA gene homologous sequenc.

Job Title: S01
Program: BLASTN
Query: EC01 ID: lcl|Query_55323(dna) Length: 308
Database: nt Nucleotide collection (nt)
Sequences producing significant alignments:

Description	Scientific name	Common name	Max taxid	Total score	Query score	E cover	Per. Value	Acc. Ident	Len	Accession
Enterobacter hormaechei strain IE2(2) 16S ribosomal RNA gene,...	Enterobacter...	NA	158836	558	558	100%	2e-154	99.35	1164	MN032348.1
Enterobacter cloacae strain IE1(2) 16S ribosomal RNA gene,...	Enterobacter...	NA	550	558	558	100%	2e-154	99.35	1164	MN032347.1
Enterobacter hormaechei strain IA3 16S ribosomal RNA gene,...	Enterobacter...	NA	158836	558	558	100%	2e-154	99.35	1160	MN006036.1
Enterobacter cloacae strain RCB714 16S ribosomal RNA gene,...	Enterobacter...	NA	550	555	555	99%	2e-153	99.35	1362	KT260926.1
Enterobacter hormaechei strain D15 16S ribosomal RNA gene,...	Enterobacter...	NA	158836	555	555	99%	2e-153	99.35	1476	KJ863539.1
Enterobacter sp. d8(2011) 16S ribosomal RNA gene, partial...	Enterobacter...	NA	943412	555	555	99%	2e-153	99.35	1497	HQ652601.1
Enterobacter sp. strain CLSEnt02 16S ribosomal RNA gene, parti...	Enterobacter...	NA	42895	553	553	100%	7e-153	99.03	980	MH021855.1
Enterobacter cloacae strain HPC-N4 16S ribosomal RNA gene,...	Enterobacter...	NA	550	553	553	99%	7e-153	99.34	717	KX456111.1
Enterobacter hormaechei strain U296 16S ribosomal RNA gene,...	Enterobacter...	NA	158836	553	553	100%	7e-153	99.03	1324	KT345643.1

The number of colony-forming units is 2×10^7 mg/ml CFU concentrations. A Gram stain was used to identify these colonies after they had been cleansed and it revealed red bacilli. The name N.L. gen. Nov. hormaechei honors Estenio Hormaeche, a scientist from Uruguay who co-founded and characterized the genus Enterobacter with P.R. Edwards and Hormaeche (1960). Nimbalkar *et al.,* (2022) reported that *E. hormaechei* is also known to produce siderophores. The target 16S rRNA fragment was found in the gDNA of the three *E. hormaechei* isolates that demonstrated Safe-Green (353 bp) amplification, as shown in Fig 2. The isolated strain utilized in this study is listed in NCBI-Gen Bank submissions as *E. hormaechei* strains IE1, 2and 3and the 16S rRNA gene sequence is designated by the accession number MN032348.1.

Sequences of the type strain's 16S rRNA and strains of *E. hormaechei* and *E. cloacae* are 100–99% similar. To determine the links between the detected Enterobacter sp. strain and the published sequences with the highest homologies, a phylogenetic analysis was performed. The linked taxa clustered together in the bootstrap test with 101 repetitions. The strain of Enterobacter sp. can be categorized as *E. hormaechei* based on this phylogenetic study (Table 2).

E. hormaechei strains are described as fermentative, non-pigmented, gram-negativeand oxidase-negative rods that share traits with members of the genus Enterobacter and the family Enterobacteriaceae (Table 1). Table 1 provides a summary of the isolate's biochemical characteristics. The microscopic appearance and morphology of colonies on growth media, as well as biochemical studies, were used to determine that the isolated bacterial strain belonged to the family Enterobacteriaceae, which displayed a pattern similar to *E. hormaechei* as O'Hara *et al.,* (1989) reported and Hoffmann *et al.,* (2005) indicated motility in SIM-agar at 37°C. The results of the biochemical assays supported the diagnosis of E. hormaechei. Even still, the urease result was uncommon because 99% of the strains were reported to have positive outcomes.

The development of molecular techniques enables the study of a sizable portion of the bacterial genome.

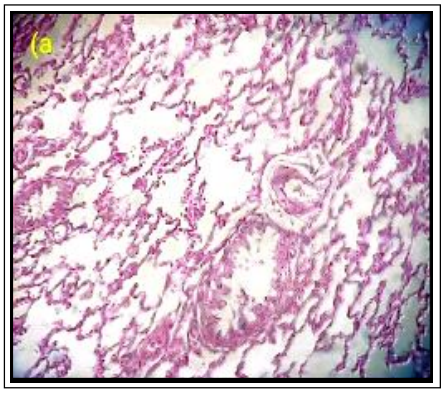

Fig 3: (a) A histological section of the lung of a non-infected rat shows the normal structure of the bronchiole and alveoli (H and E stain 100X).

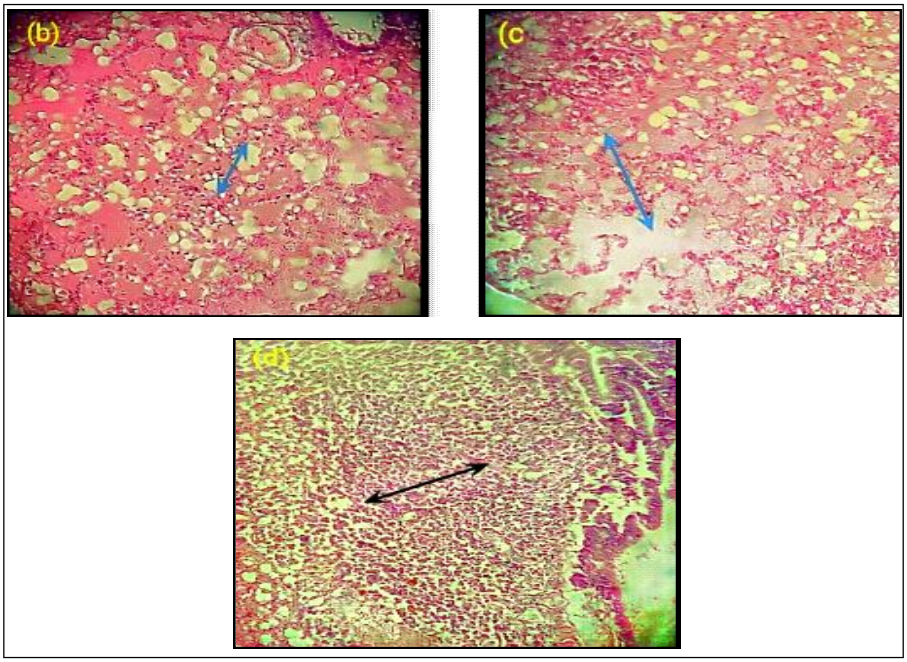

Fig 3: (b) A histological section of the lung of an infected rat with *Enterobacter hormaechei*. Shows the thickening of inter alveolar septa with edema and the infiltration of mononuclear cells 100X. (c) Diffuse edema filling the alveolar lumen with the thickening of interstitial tissue. (d) The proliferation of bronchial-associated lymphoid tissue (200X) (H and E staining).

Because the PCR technique is faster at identifying the type of bacteria present in milk samples, (Vatalia *et al.* 2020) utilized it to identify *Enterobacter* spp. as one of the leading mastitis pathogens. The most often used molecular technique in taxonomic investigations is the analysis of variable sections of bacterial 16S rRNA gene sequences according to Ibal *et al.,* (2019). Reclassification of bacteria may be feasible by using this molecular sequence to create phylogenetic trees based on changes in nitrogenous bases between species. Similar finding was reported Sabaa *et al.* (2023) and Jenkins *et al.* (2012).

Three to five days after colonization, the pathogenesis causes rats to exhibit disease signs, which are preceded by weight loss. The rats will adopt slumped positions and exhibit less activity and reactivity to stimulation, including handling, as their condition worsens. Since blood was used to isolate the germs, sepsis and/or pneumonia are usually indicators of sickness at this stage.

Histopathological examination of infected rat lung revealed thickening of interalveolar septa with edema and infiltration of mononuclear cells, as well as diffuse edema filling the alveolar lumen with thickening of interstitial tissue and proliferation of bronchial-associated lymphoid tissue (Fig 3 (b, c and d), respectively, compared with Fig 3 (a) This demonstrates the lung's typical anatomy.

Moreover, the intestinal histopathological section exhibits destruction and necrosis of intestinal villi with infiltration of lymphoplasmacytic cells in lamina properia (Fig 4).

While uterine histopathological sections show widespread hemosiderin pigment accumulation in the endometrial layer, diffuse infiltration of inflammatory cells with vacuolation of epithelial cells in the endometrium and myometrium layers, as well as focal necrosis of endometrium epithelium cells with degeneration of endometrium glands, respectively [Fig 5 (a, b and c)] and additionally the presence of atresia of oviduct epithelia (Fig 6).

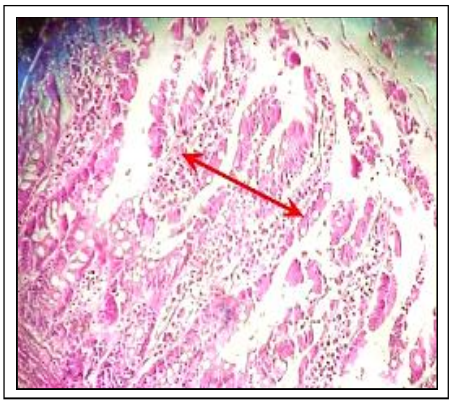

Fig 4: A histopathological section of the intestine of an infected rat with *Enterobacter hormaechei* shows destruction and necrosis of intestinal villi with infiltration of lymphoplasmacytic cells in the lamina properia (”↔) (H and E stain 200X).

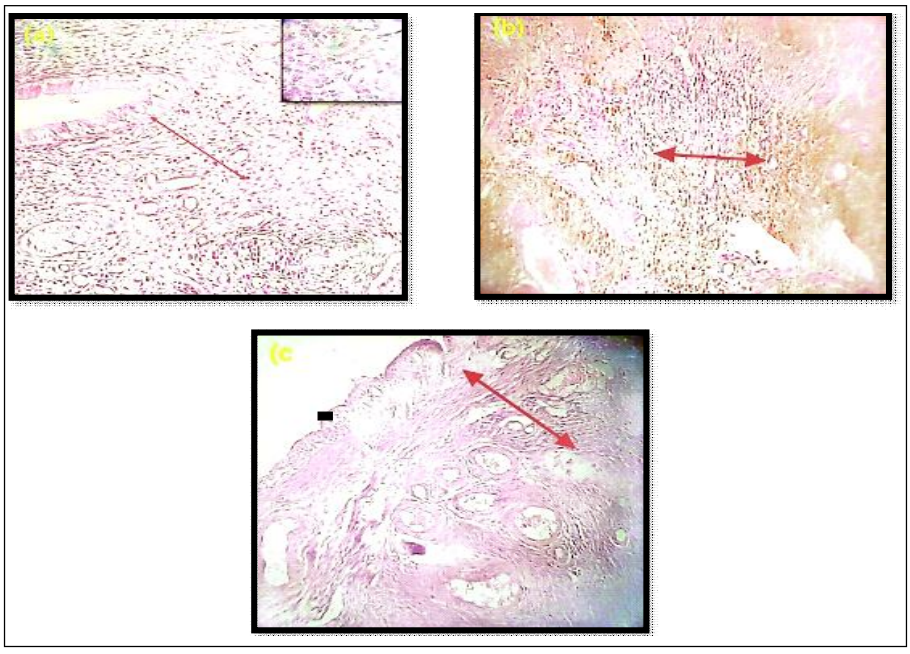

Fig 5: (a) A histopathological section of the uterus of an infected rat with *Enterobacter hormaechei* demonstrates that inflammatory cells have infiltrated widely in the endometrium and myometrium layers with vacuolation of epithelial cells (200X). (b) histopathological section of the uterus of an infected rat with *Enterobacter hormaechei* shows diffuse infiltration of inflammatory cells with deposition of hemosiderin pigment in the endometrial layer (100X); (c) histopathological section of the uterus of an infected rat with *Enterobacter hormaechei* shows focal necrosis of endometrium epithelial cells with degeneration of endometrium glands (200X) (H and E staining).

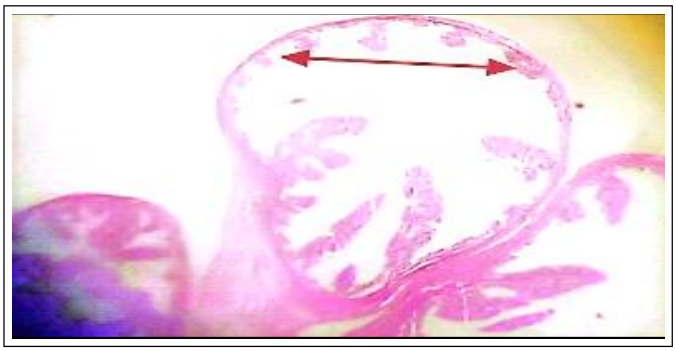

Fig 6: A histopathological section of the oviduct of an infected rat with *Enterobacter hormaechei* shows atretic oviduct epithelia (H and E stain 100X).

Histological features of infected rats' lungs observed alveolar septum thickening and mononuclear infiltration (Fig 3) were also recorded in Hongfei *et al.* (2022) and Zhicheng *et al.,* (2020) the lungs of dead sheep, alveolar septal thickening and bronchial epithelial cell destruction, the release of mucus and the presence of neutrophils were seen. Similar to this, in Carvalho-Assef *et al.,* (2014), Lung histopathology samples that contained the *Enterobacter hormaechei* strain showed thickened alveolar septa, erythrocyte displacement and inflammatory cell invasion along the pulmonary alveolar septa.

Fig 4 the intestine of an infected rat gives destructions and necrosis like in a piglet's intestinal illness has been documented, Additionally, only one uterine infection led to the isolation of *E. hormaechei* from the uterine secretions of a dead fox, despite the fact that few investigations of *E. hormaechei* are connected to infection in animals also reported by Shan-Shan *et al.* (2017); Paauw *et al.* (2009) and Lu-Yao *et al.* (2017). *E. hormaechei* was also isolated from blood after infected rat. According to Daurel *et al.* (2009); Giammanco *et al.* (2011); Pereira *et al.* (2015); Rafferty *et al.* (2011); Sampaio *et al.* (2014) and Yang *et al.* (2018) Injuries, tissues, organsand body fluids of patients can all contain *E. hormaechei.*

CONCLUSION

The study's findings imply that the presence of *Enterobacter hormaechei* in recycled napkins is a sign of contamination and that the organism can infect an organ and cause sickness.

Conflicts of interest

Conflicts of interest by the authors are not disclosed.

REFERENCES

Abbas, Maryam, Morovat, Iraj, Jasem, Marcela, Ebrahim, Nourkhoda. (2020). The characterization of bacterial communities of oropharynx microbiota in healthy children by combining culture techniques and sequencing of the 16S rRNA gene. Microbial Pathogenesis. Jun. 143: 104115. https://doi.org/10.1016/j.micpath. 2020. 104115.

Abbas, A.A., Faraj, M.K. and Abed, A.R. (2012) Some biochemical and histopathological effects of different oral doses Ochratoxin A in male rats. Proceeding of the Eleventh Veterinary Scientific Conference. The Iraqi Journal of Veterinary Medicine. 36: special issue. (2): 182-189. https://doi.org/10.30539/iraqijvm.v36i0e.414

Abhyankar P.S., Gunjal, A.B., Kapadnis, B.P., Ambade, S.V. (2022). Potential of lactic acid bacteria in plant growth promotion. Bhartiya Krishi Anusandhan Patrika. 36(4): 326-329. https://doi.org/10.18805/BKAP374.

Blanco, MA, Negro, C, Gaspar, I, Tijero, J. (1996). Slime problems in paper and board industry. Appl Microbiol Biotechnol. 46: 203-208. https://doi.org/10.1007/s002530050806.

Boone, D.R. and Castenholz, R.W. (2001) Bergey's Manual of Systematic Bacteriology, 2nd. Vol. I. Heidelberg, Germany: Springer Verlag. https://doi.org/10.1007/978-0-387-21609-6

Carvalho-Assef, A.P., Pereira, P.S., Albano, R.M., Beriao, G.C., Tavares, C.P., Chagas, T.P., Marques, E.A., Timm, L.N., Da, Silva, R.C., Falci, D.R., *et al.* (2014). Detection of ndm-1-, ctx-m-15-and qnrb4-producing enterobacter hormaechei isolates in Brazil. Antimicrob Agents Chemother. 58:2475–6. https://doi.org/10.1128/aac.02804-13.

Daurel, C., Fiant, A.L., Bremont, S., Courvalin, P., Leclercq, R. (2009). Emergence of an enterobacter hormaechei strain with reduced susceptibility to tigecycline under tigecycline therapy. Antimicrob Agents Chemother. 53: 4953-4. https://doi.org/10.1128/aac.01592-08.

Davin-Regli, A., Bosi, C., Charrel, R., Ageron, E., Papazian, L., Grimont, P.A., Cremieux, A., Bollet, C.A. (1997). Nosocomial outbreak due to enterobacter cloacae strains with the *E. hormaechei* genotype in patients treated with fluoroquinolones. J. Clin. Microbiol. 35:1008-10. https://doi.org/10.1128/jcm.35.4.1008-1010.1997.

Gaston, M.A. (1988) Enterobacter: An emerging nosocomial pathogen. Journal of Hospital Infection. 11(3): 197-208. https://doi.org/10.1016/0195-6701(88)90098-9.

Giammanco, G.M., Aleo, A., Guida, I., Mammina, C. (2011). Molecular epidemiological survey of citrobacter freundii misidentified as Cronobacter spp. (Enterobacter Sakazakii) and enterobacter hormaechei isolated from powdered infant milk formula. Food borne Pathog Dis. 8: 517-25. https://doi.org/10.1089/fpd.2010.0719.

Hoffmann, H. and Roggenkamp, A. (2003). Population genetics of the nomenspecies enterobacter cloacae. Applied and Environmental Microbiology. 69(9): 5306-5318. https://doi.org/10.1128/aem.69.9.5306-5318.2003.

Hoffmann, H., Stindl, S., Ludwig, W., Stumpf, A., Mehlen, A., Monget, D., Pierard, D., Ziesing, S., Heesemann, J., Roggenkamp, A. and Schleifer, K.H. (2005). Enterobacter hormaechei subsp. oharaesubsp. nov., E. hormaechei subsp. hormaechei combnov. and E. hormaechei subsp. steigerwaltii subspnov., three new subspecies of clinical importance. Journal of Clinical Microbiology. 43(7): 3297-3303. https://doi.org/10.1128/jcm.43.7.3297-3303.2005

Hongfei, Kun, Li, Shiyu, Bozhen L and Lunguang. (2022). Case report of enterobacter hormaechei in sheep with respiratory disease and death. BMC Veterinary Research. 18:57 https://doi.org/10.1186/s12917-022-03157-z.

Hormaeche, E. and P.R. Edwards. (1960). A proposed genus Enterobacter. Int. Bull. Bacteriol. Nomencl. Taxon. 10:71-74. https://doi.org/10.1099/0096266x-10: 2-71. https://doi.org/10.1186/s12917-019-2207-z.

Ibal, J.C., Pham, H.Q., Park, C.E. and Shin, J.H. (2019). Information about variations in multiple copies of bacterial 16S rRNA genes may aid in species identification. PLoS ONE. 14(2): e0212090.https://doi.org/10.1371/journal.pone.0212090.

Jenkins, C., Ling, C.L., Ciesielczuk, H.L., Lockwood, J., Hopkins, S., McHugh, T.D., Gillespie, S.H. and Kibbler, C.C. (2012) Detection and identification of bacteria in clinical samples by 16S rRNA gene sequencing: Comparison of two different approaches in clinical practice. Journal of Medical Microbiology. 61(Pt 4): 483-488. https://doi.org/10.1099/jmm.0.030387-0.

Khalifa, H.O., Oreiby, A., Abd, El-Hafeez, A.A. Abd, El Latif A, Okanda, T., Kato, Y., Matsumoto, T. (2021). High beta-lactam and quinolone resistance of enterobacteriaceae from the respiratory tract of sheep and goat with respiratory disease. Animals (Basel). 11: 2258. https://doi.org/10.3390/ani11082258.

Khalifa, H.O., Oreiby, A.F., Abd, El-Hafeez, A.A., Okanda, T., Haque, A., Anwar, K.S., Tanaka, M., Miyako, K., Tsuji, S., Kato, Y., *et al.* (2020). First report of multidrug-resistant carbapenemase-producing bacteria coharboring mcr-9 associated with respiratory disease complex in pets: Potential of animal-human transmission. Antimicrob Agents Chemother. 65: e01890-20. https://doi.org/10.1128/aac.01890-20.

Krishnasree, V., Nethra, P.V., Dheeksha, J., Madumitha, M.S., Vidyaeswari, R., Lakshya, P. (2018). A pilot study on assessing the sustainability of food safety and hygienic practices in street food handling system. Asian Journal of Dairy and Food Research. 37(4): 321-325. https://doi.org/10.18805/ajdfr.DR-1381.

Lu-Yao, L.I., Liu, M.J., Teng, M.M., Wang, L, Zhang, Y.X., Liu, B.Q. (2017). Study on the biological characteristics of Enterobacter hormaechei. J. Ani. Sci. Vet. Med. 36: 1-6. https://doi.org/10.1186/s12917-019-2207-z.

Martínez, D., Rodulfo, H.E., Rodríguez, L., Caña, L.E., Medina, B., Guzman, M., Carreño, N., Marcano, D., De, Donato, M. (2014). First report of metallo-β-lactamases producing *Enterobacter* spp. strains from Venezuela. Rev. Inst. Med. Trop Sao Paulo. 56(1): 67-69. https://doi.org/10.1590/ s0036-46652014000100010.

Mezzatesta, M.L., Gona, F. and Stefani, S. (2012). Enterobacter cloacae complex: Clinical impact and emerging antibiotic resistance. Future Microbiology. 7(7): 887-902. https://doi.org/10.2217/fmb.12.61.

Morand, P.C., Billoet, A., Rottman, M., Sivadon-Tardy, V., Eyrolle, L., Jeanne, L., Tazi, A., Anract, P., Courpied, J.P., Poyart, C. and Dumaine, V. (2009). Specific distribution within the Enterobacter cloacae complex of strains isolated from infected orthopedic implants. Journal of Clinical Microbiology. 47(8): 2489- 2495. https://doi.org/10.1128/jcm.00290-09.

Nallaiah, Hemamalini, Seerappalli, Aran, Shanmugam, Ayyathurai Kathirvelpandian, Agarwal Deepak, Venkatachalam Kaliyamurthi, Eswaran Suresh, Selvaram Ezhilmathi (2022). Prevalence, Antimicrobial Resistance and Resistance Gene Cassettes Detection in Bacterial Pathogens Isolated from Freshwater Ornamental Fishes. Indian Journal of Animal Research. https://doi.org/10.18805/IJAR.B-4903

Nimbalkar, Raje, R.U., Barge, N.S., Marathe, R.J., Phatake, Y.B., Deshmukh, R.B., Dange S.S., Mane S.G., Dhawan S.S. (2022). Application of Response Surface Methodology for Optimization of Siderophore Production. Indian Journal of Agricultural Research. 56(2): 230-237. https://doi.org/10.18805/IJARe.A-5663.

O'Hara, C.M., Steigerwalt, A.G., Hill, B.C., Farmer, J.J. 3rd, Fanning, G.R., Brenner, D.J. (1989). Enterobacter hormaechei, a new species of the family enterobacteriaceae formerly known as enteric group 75. J. Clin. Microbiol. 27: 2046-9. https://doi.org/10.1128/jcm.27.9.2046-2049.1989

Paauw, A., Caspers, M.P., Leverstein-van, Hall, M.A., Schuren, F.H., Montijn, R.C., Verhoef, J., Fluit, A.C. (2009). Identification of resistance and virulence factors in an epidemic enterobacter hormaechei outbreak strain. Microbiology. 155: 1478-88. https://doi.org/10.1099/mic.0.024828-0.

Pereira, P.S., Borghi, M., Albano, R.M., Lopes, J.C., Silveira, M.C., Marques, E.A., Oliveira, J.C., Asensi. M.D., Carvalho-Assef, A.P. (2015). Coproduction of NDM-1 and KPC-2 in enterobacter hormaechei from Brazil. Microb Drug Resist. 21:234-6. https://doi.org/10.1089/mdr.2014.0171.

Poirel, L., Walsh, T.R., Cuvillier, V., Nordmann, P. (2011) Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis. 70(1): 119-123. https://doi.org/10.1016/j.diagmicrobio.2010.12.002.

Rafferty, B., Dolgilevich, S., Kalachikov, S., Morozova, I., Ju, J., Whittier, S., Nowygrod, R., Kozarov, E. (2011) Cultivation of enterobacter hormaechei from human atherosclerotic tissue. J . Atheroscler Thromb. 18:72-81. https://doi.org/10.5551/jat.5207.

Sabaa, Hilal, Hadi, Afaf, Abdulrahman, Abdulaziz, A. (2023). Enterobacter cloacae identification by 16S rRNA and rpo B gene sequencing of the Cichlidae Tilapia fish pathogen in Babel local market. Journal of Survey in Fisheries Sciences. 10(3S): 1701-1708.

Sampaio, J.L., Ribeiro, V.B., Campos, J.C., Rozales, F.P., Magagnin, C.M., Falci, D.R., da, Silva, R.C., Dalarosa, M.G., Luz, D.I., Vieira, F.J., *et al.* (2014) Detection of oxa-370, an oxa-48-related class d beta-lactamase, in enterobacter hormaechei from Brazil. Antimicrob Agents Chemother. 58: 3566-7. https://doi.org/10.1128/aac.02510-13.

Shan-Shan, W., Yun-Jia, S., Xing-Yang, C., Cheng-Wei, W., Shan-Shan, G.U., Xin, Y., Shuang, X., Jun-Wei, G.E., Hong-Yan, C. (2017). Isolation, identification and phylogenetic analysis of enterobacter hormaechei from foxes. Chin Vet Sci. 47: 768-72.

Sorelle, P.H., Belgard, W.E. (1991). The evect of recycled Wber use on paper machine biological control. TAPPI Proceedings. pp 569-575.

Southam, D.S., Dolovich, M., *et al.* (2002). Distribution of intranasal instillations in mice: effects of volume, time, body position. Lung Physiol. 282: 833-839. https://doi.org/10.1152/ajplung.00173.2001.

Suihko, M.L. and Skytta, E. (1997) A study of the microflora of some recycled fibre pulps, boards and kitchen rolls. Journal of Applied Microbiology. 83: 199-207. https://doi.org/10.1046/j.1365-2672.1997.00219.x

Suominen, I., Suihko, M.L. and Salkinoja-Salonen, M.(1997) Microscopic study of migration of microbes in food-packaging paper and board. Journal of Industrial Microbiology and Biotechnology. 19: 104-113. https://doi.org/10.1056/nejmcp031807.

Swartz, M.N. Clinical practice. Cellulitis. N. Engl. J. Med. (2004); 350: 904-12. https://doi.org/10.1056/nejmcp031807.

Vatalia, D.J., Bhanderi, B.B., Nimavat, V.R., Jhala, M.K. (2020). Isolation, Molecular Identification and Multidrug Resistance Profiling of Bacteria Causing Clinical Mastitis in Cows. Agricultural Science Digest. 41(2): 345-351. https://doi.org/10.18805/ag.D-5220.

W.G. Weisburg, 1, S.M. Barns, D.A. Pelletier, D.J. Lane. (1991). 16S ribosomal DNA amplification for phylogenetic study, PMID: 1987160 PMCID: PMC207061 doi: 10.1128/jb.173.2.697-703. https://doi.org/10.1128/jb.173.2.697-703.1991.

Wang, Z., Duan, L., Liu, F., Hu, Y., Leng, C., Kan, Y., Yao. L., Shi H. (2020). First report of enterobacter hormaechei with respiratory disease in calves. BMC Vet Res. 16:1. https://doi.org/10.1186/s12917-019-2207-z.

Xiong, Z., Wang, P., Wei, Y., Wang, H., Cao, H., Huang, H. and Li, J. (2008). Investigation of qnr and aac(6')-Ib-cr in Enterobacter cloacae isolates from Anhui Province, China. Diagnostic Microbiology and Infectious Diseases. 62(4): 457-459. https://doi.org/10.1016/j.diagmicrobio.2008.07.010.

Yang, B., Feng, Y., McNally, A., Zong, Z. (2018). Occurrence of enterobacter hormaechei carrying blandm-1 and blakpc-2 in China. Diagn Microbiol Infect Dis. 90:139-42. https://doi.org/10.1016/j.diagmicrobio.2017.10.007

Zhicheng Wang, Lisha Duan, Fei, Liu, Yun, Hu, Chaoliang, Leng, Yunchao Kan, Lunguang Yao and Hongfei Shi. (2020). First report of enterobacter hormaechei with respiratory disease in calves. Wang *et al.* BMC Veterinary Research. 16: 1.