Iran. J. Ichthyol. (Special Issue 2023): 303-309

P-ISSN: 2383-1561; E-ISSN: 2383-0964
http://www.ijichthyol.org

Research Article

Isolation, identification, and antibiotic sensitivities of bacteria from sheep and human gallbladders

Ban Sahib ABDUL-NABI AL-NASIRY[1], Noor Muthanna MAHMOOD[1], Mohammed M. DAKHEEL[2*]

[1]*Zoonosis Diseases Research Unit, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.*

[2]*Department of Public Health, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.*

[*]*Email: m.m.dakheel@covm.uonbaghdad.edu.iq*

Abstract: This investigation examined bacterial types in sheep gallbladders and human gallbladders with cholecystitis. Samples were collected to isolate and describe bacterial types, identify their relationship to cholecystitis, detect common germs in humans and sheep, and assess the sensitivity and resistance of bacterial isolates to antibiotics. The study found 56 sheep gallbladder isolates classified into nine types, including *Staphylococcus aureus* (12.8%), *Streptococcus* spp., *Salmonella typhimurium* and *Pseudomonas aeruginosa* (4.2%), *E. coli* (28.5%), *Klebsiella* spp., *Listeria monocytogenes* and *Pasteurella* spp. (5.7%), and *Proteus* spp. (8.5%). In contrast, in 78 human gallbladder isolates were found nine types, including *S. aureus* (18.3%), *Listeria monocytogenes* and *Salmonella typhimurium* (15%), *E. coli* (36.6%), *Klebsiella* spp. (8.3%), *Proteus* (11.6%), and *Streptococcus* spp. and *Pasteurella* spp. As a result, antibiotic isolates from sheep showed high sensitivity to antibiotics such as Ciprofloxacin, Rifampin, Ampicillin, Imipenem, and Gentamicin. In contrast, human isolates showed increased sensitivity to Imipenem, Ciprofloxacin, Vancomycin, Trimethoprim, and Rifampin, while the samples were highly resistant to Chloramphenicol, Erythromycin, Gentamicin, Ampicillin, and Cefotaxime. These findings suggest that antibiotic sensitivity and resistance are crucial factors in antibiotic resistance.

Keywords: Zoonotic bacteria, Sheep, Human gallbladder, Antibiotic resistance.

Citation: Abdul-Nabi Al-Nasir, B.S.; Mahmood, N.M & Dakheel, & M.M. 2023. Isolation, identification, and antibiotic sensitivities of bacteria from sheep and human gallbladders. Iranian Journal of Ichthyology (Special Issue 1): 303-309.

Introduction

Biliary system infections include acute cholecystitis and cholangitis, frequently caused by predisposing conditions such as bacteremia (Ballal et al. 2019) and the ossification of bile's natural dissolved components, resulting in persistent blockage (Capoor et al. 2008; Strohäker et al. 2021). Cholelithiasis can affect any species of field animal; although it is most common in ruminants (Raoofi et al. 2012). It also affects humans, especially adults. Daily, the liver secretes up to a liter of bile into the digestive system; bile exposure is a significant risk (Begley et al. 2005). Bile salts are used by intestinal bacteria as environmental signals, as well as nutrients and electron acceptors in some situations (Urdaneta & Casadesús 2017).

Bacteria of the intestine participate in bile acid oxidation, and epimerisation can alter hepatic circulation and cause gallstone development (Grigor'eva & Romanova 2020). Certain bile-resistant microorganisms can colonize the hepatobiliary system; one notable example is *Salmonella enterica* serovars *Typhi* and *Paratyphi,* persistent gall bladder infections, especially in countries where enteric fever is widespread, such as India. Enteric fever persists for years after recovery,

increasing the risk of hepatobiliary cancer (Capoor et al. 2008; Al-Nasiry 2011; Urdaneta & Casadesús 2017). In addition, *Enterococci* accounted for 23%, *Pseudomonas* 1%, and *Salmonella typhi* 2-5%; other gram-negative bacteria, especially *Escherichia coli,* reached 41.1% (Eslami et al. 2007; Zghair et al. 2013Al-Nasiry 2020). *Escherichia coli* is a bile-resistant bacterium regularly isolated from the gallbladder and bile of both animals and humans (Begley et al. 2005; Dakheel et al. 2023). *Lactobacillus* sp. can thrive on bile in the small intestine, even at concentrations of 5% (Whitehead et al. 2013).

The novel bile exclusion system plays a role in excluding the effect of bile and determining the virulence of *listeria* and the reproduction of bacteria within a concentration of bile acids that does not exceed 20mM. Concentrations of these acids in the intestine are at a rate of 5 mM, so it protects bacteria through this system and enhances the presence of bacteria in the small intestine and their stay for a long time (Sleator et al. 2005; Kawakib Ibraheem 2006); *Listeria monocytogenes* strain can survive in high bile concentrations (Al-Khatib et al. 2007). A change in the motility of the gallbladder constitutes one of the promoters of gallbladder stone development through immunomodulation processes; the significance of bacterial infection in the etiology of pigmented gallstones. Later, it was established that alterations in the gut microbiota are an etiological cause of cholesterol gallstones (Capoor et al. 2008; Grigor'eva & Romanova 2020).

Human immunodeficiency virus cholangiopathy, biliary parasites, and primary sclerosing cholangitis are all general causes of cholecystitis. Other reasons involve severe burns, trauma, major surgery, diabetes, unpublic bacterial infections of Gallbladder like *Vibrio cholera*, and other systemic diseases such as tuberculosis and syphilis. Furthermore, acute cholangitis can cause septicemia and consequences: perforation, emphysema, gangrene, and chronic cholecystitis, even with rapid diagnosis and treatment (Capoor et al. 2008). When the bile duct is obstructed, the ductal pressure rises, allowing bacteria to multiply and spread, which is the most prevalent cause of acute cholangitis, especially Gram-negative bacteria, while Gram-positive and anaerobic bacteria are unusual causes; viruses and fungi are scarce (Capoor et al. 2008). Chronic cholecystitis results from gallstones, occurring in 4% of cases. Finally, gallstones are the most common cause of obstruction in 80% of patients (Al-Nasiry 2020; Strohäker et al. 2021). Cholelithiasis in the gallbladder is not clinically significant and does not cause symptoms unless it migrates or blocks the extra-hepatic bile channels (Raoofi et al. 2012).

They were currently using 16S rRNA gene-based sequencing to examine the biliary microbiome in gallstone sufferers (Ye 2020; Qasim & Al-Nasiry 2021). Some people in African countries like Sudan employ raw bile from slaughtered ruminants as an appetizer in some foods (Eldirdery & Elobeid 2007). Antibiotic-resistant microorganisms are transferred directly from animals to humans. Resistance to one antibiotic in a class frequently leads to resistance to all medications in that class, exacerbating the problem in the future. To reduce this health danger, enteric bacteria must be identified in food. Antibiotics are used in food-producing animals to cure or prevent infections or to stimulate development; animals may be a source of foodborne resistant bacteria (Abbas et al. 2011; Zare et al. 2014; Al-Nasiry 2022). Therefore, this work aimed to isolate and describe bacterial types, identify their relationship to cholecystitis, detect common germs in humans and sheep, and assess the sensitivity and resistance of bacterial isolates compared to antibiotics.

Materials and Methods

Isolation and identification of bacterial isolates

Sample collection: One hundred thirty gallbladder samples were collected from slaughtered sheep (n=70) and humans (n=60) exposed to cholecystectomy in Baghdad City from October 2017 to November 2019. All models were transferred in

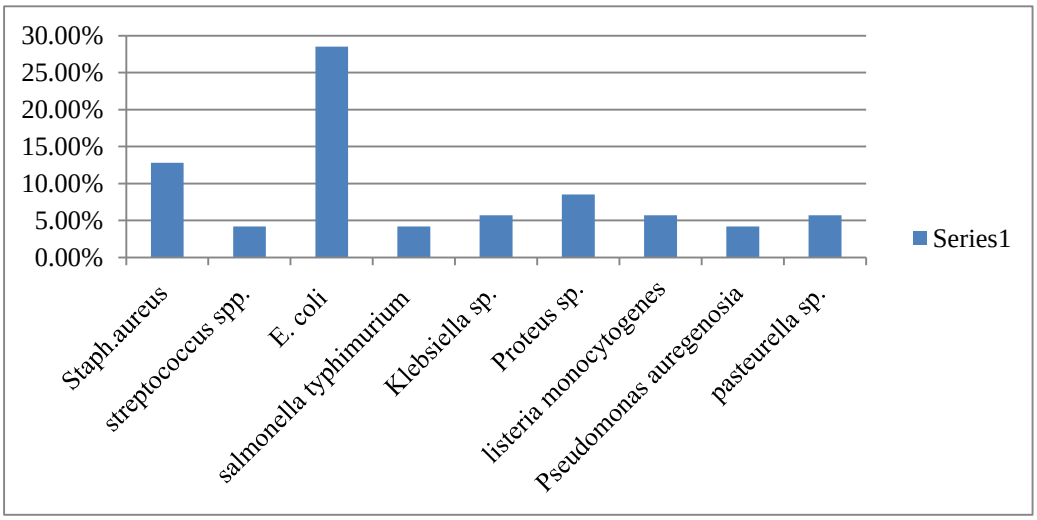

Fig.1. Percentage of bacteria isolated from sheep gallbladder.

sterile cooled bags to the laboratory to perform the necessary tests, and bile samples were stored at −20°C until analysis.

Sample inoculation: All samples were placed at 4°C for a week and centrifuged at 3000rpm for 20 min. A 1ml of bile salt pellets inoculated into 9ml of enrichment broth trypticase soy broth with yeast extract, incubated at 37°C for 24 hours. After that, 0.1 ml from the cultured broth was spread on selective and differential media such as Blood agar, MacConkey agar, Nutrient agar, Eosin methylene blue agar (EMB), Salmonella-Shigella agar (SS), and Mannitol salt agar. The colonies were studied after 24-48 hours and incubated at 37°C. Then, the samples were re-cultured after 10 days, and Biochemical tests confirmed the results. Finally, the samples were frozen at 70°C in BHI broth containing 20% glycerol for the susceptibility test.

Bacterial diagnosis: According to Quinn et al. (2022), microorganisms were identified and characterised through a direct microscopic examination using Gram stain and observation of bacterial shapes, as well as a macroscopic examination of colonies on their specific media and final confirmation by diagnostic characteristic biochemical tests.

Antibiotic susceptibility test: The Kirby-Bauer disc diffusion technique was used to determine the resistant isolates. The inhibition zone was measured and categorised as resistant according to the CLSI criteria (Al-Khatib et al. 2007). Species of isolates were tested against (10 routine and practical antibiotics). The susceptible and resistant findings were made based on the diameter of the clear zone surrounding disks concerning the antibiotic standard table. Ampicillin (AM) (10µg/disc), gentamicin (GM) (10µg/disc), Ciprofloxacin (CF) (5µg/disc), Rifampin (RA) (5µg/disc), Imipenem (IPM) (10µg/disc), Erythromycin (E) (10µg/disc), Vancomycin (VA) (30µg/disc), Chloramphenicol (CP) (30µg/disc), Trimethoprim (TMP) (5µg/disc), and Cefotaxime (CTX) (5µg/disc). The disks were acquired from Mast Group Ltd. (England). Multi-antibiotic-resistant isolates were defined as those resistant to four or more antibiotics.

Results

The results showed in the sheep gallbladder samples the presence of 56 bacterial isolates, many of them contained mixed infection classified into 9 types including *S. aureus* (12.8%), *Streptococcus* spp., *Salmonella typhimurium and Pseudomonas aeruginosa* (4.2%), *E. coli* (28.5%), *Klebsiella* spp., *listeria monocytogenes,* and *Pasteurella* spp. (5.7%), and *Proteus* spp. (8.5%) (Fig. 1). Moreover, the results showed in the human gallbladder samples the existence of 78 isolates that many samples contained mixed infection divided into 9 types, including

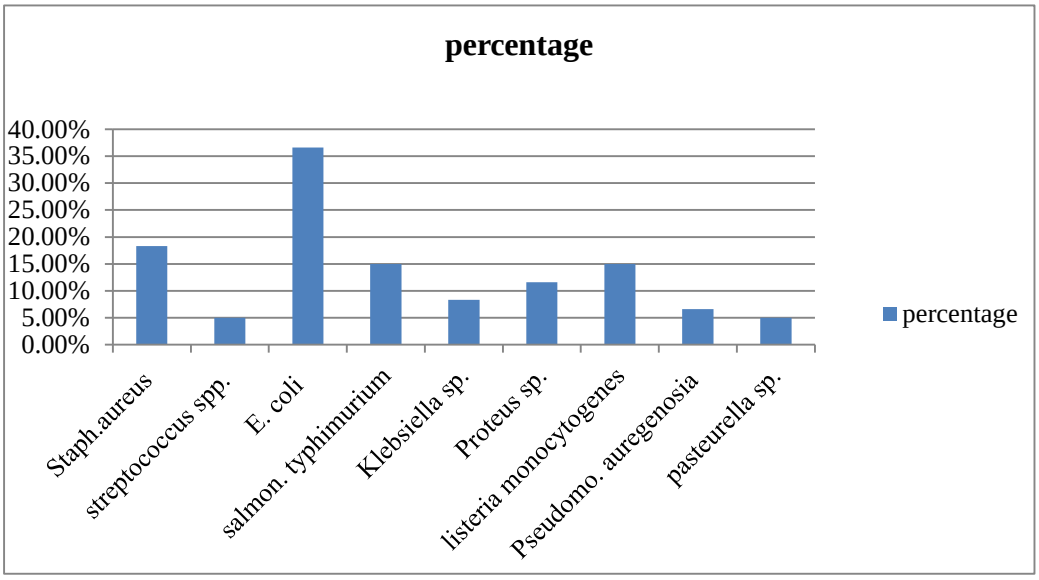

Fig.2. Percentage of bacteria isolated from human gallbladder.

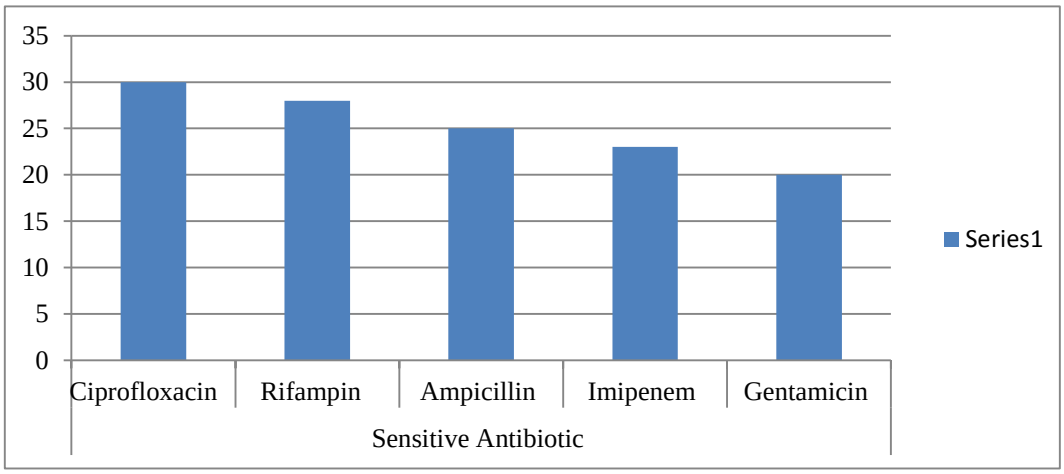

Fig.3. Sensitivity of bacterial isolates to antibiotics in sheep.

S. aureus (18.3%), *listeria monocytogenes* and *Salmonella typhimurium* (15%), *E. coli* (36.6%), *Klebsiella* spp. (8.3%), *Proteus* spp. (11.6%), *Pseudomonas aeruginosa* (6.6%), *Streptococcus* spp. and *Pasteurella* spp. (5 %) (Fig. 2).

In terms of antibiotic sensitivity, most sheep isolates exhibited high sensitivity to Ciprofloxacin (CF), Rifampin (RA), Ampicillin (AM), Imipenem (IPM), and Gentamicin (GM), respectively (Fig. 3). In contrast, other isolates were resistant to the antibiotics, such as Erythromycin (E), Vancomycin (VA), chloramphenicol (CP), Trimethoprim (TMP) and Cefotaxime (CTX) sequentially.

Antibiotic data for human isolates indicated that most of the isolates were extremely sensitive to antibiotics, including Imipenem (IPM), Ciprofloxacin (CF), Vancomycin (VA), Trimethoprim (TMP) and Rifampin (RA) sequentially (Fig. 4). Some of the isolates showed significant resistance to Chloramphenicol (CP), Erythromycin (E), Gentamicin (GM), Ampicillin (AM), and Cefotaxime (CTX), respectively (Fig. 4).

Discussion

The current results of bacterial isolation of sheep gallbladder samples showed the most common gram-positive bacteria were *S. aureus* (12.8%), followed by *Listeria monocytogenes* (5.7%), then *Streptococcus*

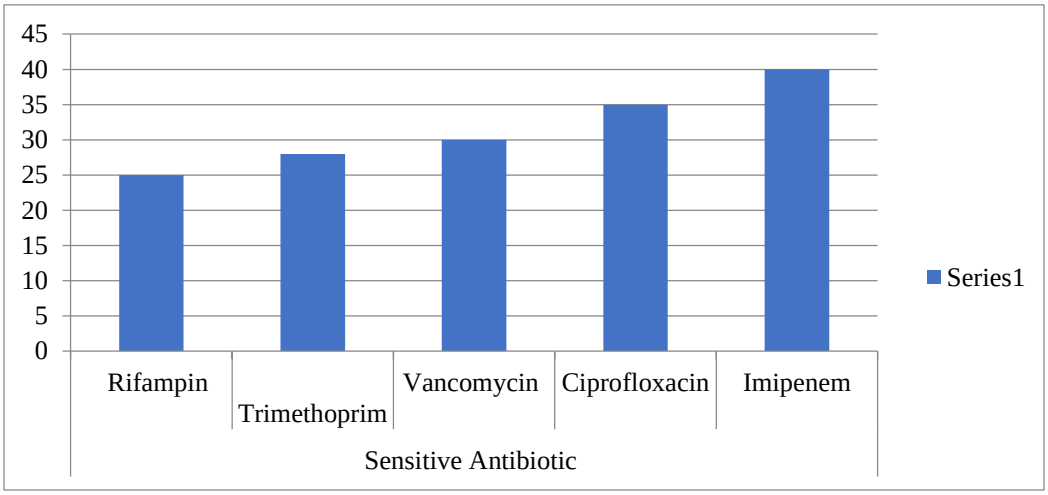

Fig.4. Sensitivity of bacterial isolates to antibiotics in human.

spp., (4.2%), that these findings are in agreement with the results of Al-Nasiry (2020) and Al-Ali et al. (2018).

As for gram-negative bacteria, *E. coli* had the highest percentage (28.5%), followed by *Proteus* spp. (8.5%), *Klebsiella* spp. and *Pasteurella* spp. (5.7%), as for the proportion of *Salmonella typhimurium* and *Pseudomonas aeruginosa* (4.2%), similar to the results of Eldirdery & Elobeid (2007) and Zare et al. (2014). For *E. coli*, our results did not correspond with the findings of Cetin et al. (2020), who found that *S. typhimurium* was the highest maybe due to environmental differences. The results of bacterial isolation of human gallbladder samples showed the most common gram-positive bacteria were *S. aureus* (18.3%), followed by *Listeria monocytogenes* (15%), then *Streptococcus* spp. (5%) disagree with the findings of Ballal et al. (2019) and Al-Nasiry (2020), where they found that *Enterococci* were the highest percentage. As for gram-negative bacteria, the most frequent were *E. coli* (36.6%), followed by *S. typhimurium* (15%), then *Proteus* spp. (11.6%), *Klebsiella* spp., (8.3%), *P. aeruginosa* (8.3%), and *Pasteurella* spp. (5%), similar to the results of Suh et al. (2021) and Ballal et al. (2019).

In the current study, Enterobacteriaceae were primarily isolated from gallbladder samples, which may be due to the use of contaminated food and water, as it is one of the opportunistic bacteria widely spread in nature. *Escherichia coli* is a bile-resistant bacterium regularly isolated from the gallbladder and bile of both animals and humans in the presence of high amounts of bile extract. Also, *Salmonellae* may invade the gallbladder, which has extraordinarily high bile contents; *L. monocytogenes* can tolerate bile and use it as a signal to boost infection and virulence even in bad conditions, such as pH variations, low oxygen levels, food deficiency, and increased osmolarity is all possible survival barriers (Begley et al. 2005). A study demonstrated the significance of bacterial infection in the etiology of pigmented gallstones; then, multiple studies established that alterations in the gut microbiota are also an etiological cause of cholesterol gallstones. Gallstones are mainly caused by abnormal gallbladder motility, abnormal cholesterol, and bile acid metabolism and release (Grigor'eva & Romanova 2020).

The results of the present study on antibiotic sensitivity in sheep showed that most isolates were susceptible to Ciprofloxacin (CF), Rifampin (RA), Ampicillin (AM), Imipenem (IPM), and Gentamicin (GM) sequentially, and corresponded with Kebede et al. (2016) to sensitivity to (GM), and differed from the findings of Zare et al. (2014) to the same antibiotics, also not compares with Cetin et al. (2020) to (CF). Based on the results of the current study regarding the sensitivity to antibiotics in humans,

they showed sensitivity to Ciprofloxacin and Vancomycin that agreed with the findings of Capoor et al. (2008) and disagreed with the results of Al-Nasiry (2002) for the same antibiotics. Also, different results with Capoor et al. (2008) in the resistance to Gentamicin and Erythromycin were observed compared to our findings which are similar to the results of Eslami et al. (2007) in the resistance to Chloramphenicol and Gentamicin. The current findings were resistant to Ampicillin and Chloramphenicol, which disagreed with the results of Capoor et al. (2008).

The increase in bacterial resistance to antibiotics has been recorded globally due to the excessive and wrong use of antibiotics in animal feed and their collection in food products and, thus, the emergence of allergic diseases to antibiotics in humans and animals. The resistance of bacteria to antibiotics may be due to the production of the enzyme beta-lactamase (Kang et al. 2020; Al-Nasiry 2022). Moreover, the resistance of bacteria to antibiotics is transmitted to humans through direct contact, and the presence of germs in meat products in addition to direct excretion from animals (Al-Jboriy et al. 2020; Zare et al. 2014; Dakheel et al. 2023).

Conclusion

The toxic effects of bile secretion accelerate once they depart the gallbladder and reach the anterior region of the small intestine, referred to as the new mechanism for excluding the bile effect. This illustrates how the link between the gut and biliary microbiota may be deduced by looking at the patient's biliary microbiota changes. To prevent the use of inappropriate antibiotics in the treatment of bacterial illnesses in humans and animals, medications should be carefully chosen.

References

Abbas, M.S.; AL-Jebouri, A.J. & Al-Nasiry, B.S.A.N. 2011. Studying the sensitivity of bacteria isolated from respiratory tract infections to peanut and various antibiotics. Eng. and Technology Journal 29(16): 1-10.

Al-Ali, H.J.; Abd Al-Rodhan, M.; Al-Hilali, S.A.; Al-Charrakh, A.H.; Al-Mohana, A.M. & Hadi, Z.J. 2018. Molecular detection of serotype groups of *Listeria monocytogenes* isolated from gallbladder of cattle and sheep in Iraq. Veterinary World 11(2018): 431-436.

Al-Jboriy, K.A.; Al-Nasiry, B.S.A.N. & Alrammah, H.S.A.A. 2010. Study of the sensitivity of some pathogenic isolated from respiratory infection in human against some plant extracts. Al-Anbar Journal of Veterinary Sciences 3(2): 103-108.

Al-Khatib, G.M.; Alwan, M.J. & Al-Jebouri, N.M. 2007. The effect of heat and cold on the *L. monocytogenes* in the Milk. The Iraqi Journal of Veterinary Medicine 31(2): 30- 38.

Al-Nasiry, B.S.A.N. 2011. Isolation and Identification of bacterial isolates from ear infection and their sensitivity to usual antibiotics in human and dogs. The Iraqi Journal of Veterinary Medicine 35(1): 159- 166.

Al-Nasiry, B.S.A.N. 2020. Detection of bacterial contamination in filled and dried biscuit products of Young Children. Annals of Tropical Medicine and Public Health 23(S16): 231-602.

Al-Nasiry, B.S.A.N. 2022. Pathological changes of *salmonella Typhimurium* and treatments with some extracts of *Cinnamomum zeylanicum* and comparing with chloramphenicol in mice. Biochemical and Cellular Archives 22(2): 3719- 3925.

Ballal, M.; Shenoy, P.A.; Rodrigues, G.S.; Devadas, S.M.; Shetty,V.; Bangera, S.R.; Asha, R.; Rajini, K.V. & Shrilaxmi, M.S. 2019. Biliary tract infections and their Microbiological Spectrum- A study from coastal region of Southern India. Revista Infectio 23(3): 253-258. Strohäker, J.; Wiegand, L.; Beltzer, C.; Königsrainer, A.; Ladurner, R. & Meier, A. 2021. Clinical presentation and incidence of anaerobic bacteria in surgically treated biliary tract infections and cholecystitis. Antibiotics 10(71): 1-11.

Begley, M.; Gahan, C.G.M. & Hill, C. 2005. The interaction between bacteria and bile. FEMS Microbiology Reviews 29(4): 625-651.

Capoor, M.R.; Nair, D.; Rajni, Khanna, G.; Krishna, S.V.; Chintamani, M.S. & Aggarwal, P. 2008. Microflora of bile aspirates in patients with acute cholecystitis with or without cholelithiasis: A tropical experience. The Brazilian Journal of Infectious Diseases 12(3): 222-225.

Cetin, E.; Temelli, S. & Eyigor, A. 2020. Nontyphoid *Salmonella* prevalence, serovar distribution and antimicrobial resistance in slaughter sheep. Food Science of Animal Resources 40(1): 21-33.

Dakheel, M. M., Al-Nasiry, B.S.A.N. & Abdal-Rudha, A.M. 2023. Assessment of various concentrations of tannin extracts on pathogenic bacteria isolated from beef compared to antibiotic sensitivities. Iranian Journal of Ichthyology 10: 165-171.

Eldirdery, M.M. & Elobeid, E.A. 2007. Isolation, characterization and sensitivity test of organisms found in bile obtained from sheep and cattle. Sudan Journal of Medical Sciences 2(3): 157- 160.

Eslami, G.; Nowruzi, J.; Fllah, F.; Goudarzi, H.; Hakemivala, M. & Jahangiri, S. 2007. Detection of bacteria responsible for gallbladder inflammation and gallstones. Iranian Journal of Clinical Infectious Diseases 2(3): 139-141.

Grigor'eva, I.N. & Romanova, T.I. 2020. Gallstone disease and microbiome. Microorganisms 8(6); 835.

Kang, J.; Hossain, A.; Park, H. C.; Kim, T.W. & Jeong, S.H. 2020. Physiological influence of tylosin tartrate overdose in vivo in rats. Acta Veterinaria Brno 89(3): 283-290.

Kawakib Ibraheem, A.Z. 2006. The role of gallbladder in the pathogenesis of *Listeria monocytogenes* in the natural and experimental infections in mice. A thesis Submitted to the College of Veterinary Medicine, University of Baghdad, in Partial fulfilment of requirement for the degree of Master of Science in Veterinary Medicine/Microbiology.

Kebede, A.; Kemal, J.; Alemayehu, H. & Mariam, S.H. 2016. Solution, identification, and antibiotic susceptibility testing of *Salmonella* from slaughtered bovine and ovine in Addis Ababa Abattoir Enterprise, Ethiopia: A Cross-Sectional Study. International Journal of Bacteriology; Article ID 3714785, 1-8.

Qasim, D.A. & Al-Nasiry, B.S.A.N. 2021. Detection of OMP31 and 16SrRNA gene encoding of *Rubella melitensis* from locally mastitis goat's milk by PCR. Indian Journal of Ecology 48(15): 233-237.

Quinn, P.I.; Markey, B.K.; Carter, M.E.; Donnelly, W.J.C. & Leonard, F.C. 2002. Veterinary microbiology and microbial disease. Oxford, UK: Blackwell Science Ltd.

Raoofi, A.; Shahraki, A.R.; Namjoo, A. & Momtaz, H. 2012. A survey on prevalence and pathological findings of gallstones in Lori-Bakhtiari sheep in Iran. The Scientific World Journal 2012, 1- 4. ID 524607.

Sleator, R.D.; Wemekamp-Kamphuis, H.H.; Gahan, C.G.M.; Abee, T. & Hill, C. 2005. A PrfA-regulated bile exclusion system (BilE) is a novel virulence factor in Listeria Monocytogenes. Molecular Microbiology 55(4): 1183-95.

Suh, S.W.; Choi, Y.S.; Choi, S.H.; Do, J.H.; Oh, H.C.; Kim, H.J. & Lee, S.E. 2021. Antibiotic selection based on microbiology and resistance profiles of bile from gallbladder of patients with acute cholecystitis. Scientific Reports 11(1): 2969.

Urdaneta, V. & Casadesús, J. 2017. Interaction between Bacteria and bile salts in the gastrointestinal and hepatobiliary tracts. Frontiers in Medicine 4(2017): 1-13.

Whitehead, K.; Versalovic, J.; Roos, S. & Britton, R.A. 2008. Genomic and genetic characterization of the bile stress response of probiotic *Lactobacillus reuteri* ATCC 55730. Applied and Environmental Microbiology 74(6): 1812-1819.

Ye, C.; Zhou, W.; Zhang, H.; Miao, L. & Lv, G. 2020. Alterations of the bile microbiome in recurrent common bile duct stone. BioMed Research International 2020, ID 4637560, 1-7.

Zare, P.; Ghorbani-Choboghlo, H.; Jaberi, S.; Razzaghi, S.; Mirzae, M. & Mafuni, K. 2014. Occurrence and antimicrobial resistance of *Salmonella* spp. and *Escherichia coli* isolates in apparently healthy slaughtered cattle, sheep and goats in East Azarbaijan Province. International Journal of Enteric Pathogens 2(1): e15451.1-4

Zghair, Z.R.; Hamzah, A.M. & Alwan, M.J. 2013. Histopathological study for the effect of ethanolic extract of *Sonchus oleraceus* on *Escherichia coli* bacteria in mice. The Iraqi Journal of Veterinary Medicine 37(2): 164- 171.