E. coli heat-stable enterotoxin 1 may be a virulence marker ofisolates pathogenic for these animals (Osek, 2003).

The EAST1 gene was also detected in human ETEC isolated from patientswith diarrhea and was mainly found among strains possessing major adherence factors such as colonization factor antigens (CFA) I and II (Yamamoto and Echeverria, 1996). These authors have alsodemonstrated that the genes for EAST1 and CFA are located on the same plasmidwhereas in porcine ETEC strains the *astA* and F4 virulence factors are carried on separate plasmids (Yamamoto and Nakazawa, 1997). Moreover, the EAST1 gene sequence of porcine F4-positiveETEC isolates was different from that of human CFA+ ETEC strains (Yamamoto *et.al*., 1997). On the otherhand, the pig origin EAST1 gene sequence is identical to that of strain O42 pathogenicfor human volunteers (Yamamoto and Echeverria, 1996).

Recently, Frydendahl, 2002 analyzed 563 *E. coli*isolates from 503 pigs with post weaning diarrhea and showed a high frequency (65.8%) of strains possessing the *astA*gene. The author also observed a close correlation between the presence of theEAST1 toxin marker and F4 fimbrial as well as LTI and STII enterotoxin genes. (Choi *et al.,* 2001) analyzed 720 *E. coli* strains isolated from piglets with entericcolibacillus's for the presence of the *astA*gene and again a close association of theEAST1 gene with the F4 fimbrial marker was found.

In conclusion, the results of the present work indicate that the *astA* gene is widely distributed among *E. coli* strains isolated from patients with gallstone. Therefore, EAST1 toxinmay represent an additional determinant playing a role in the pathogenesis of *E. coli*. Moreover, the presence of the *astA*gene makes the porcine *E. coli* strains similar to human EAST1-positive isolates that are potentiallyable to induce diarrhea (Osek, 2003).

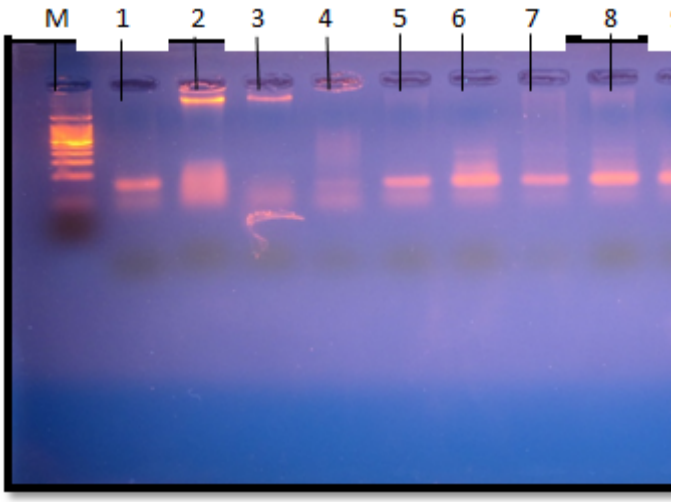

Figure 1: Agarose gel electrophoresis presenting the PCR results obtained with *astA* primers. Lane M, molecular mass marker (100 – 1500 bp range); lanes 1, 5, 6,7,8,9 *astA*-positive*E. coli*; lanes 2,3,4,10,11,12 *astA*-negative *E. coli*

Detection of *uidA*gene

PCR identification of the *uidA*gene revealed that among 50 *E. coli* isolatesanalyzed, 43 (86 %) isolates were positive as determined by the presence ofthe 1487bp amplified product (figure2). The remaining 7 (14%) strains testedwith the PCR method for the *uidA*marker did not generate a PCR amplicon of 1487bp or any other size (table 3).

beta-glucuronidase (GUD) is an inducible enzyme that is encoded by the *uidA*gene in *E. coli*(Jefferson,*et.al.*1986). About 94% of *E. coli* strains andsome *Shigella*spp. (44%) and *Salmonella* spp. (29%) appearto be the only members of the family *Enterobacteriaceae*that produce GUD, except for the pathogenicenter hemorrhagic*E. coli* of serotype 0157:H7, which is4-methylumbelliferyl-beta -D-glucuronide(MUG) negative (Feng *et.al.*1991).

Bacterial beta-glucuronidase causes deconjugation of bilirubin diglucuronide resulting in the precipitation of calcium bilirubinate, which contributes to biliary sludge and stone formation. This processes attributed to enzyme activity produced by the aerobic *Enterobacteriaceae* such as *E. coli* and *Klebsiella* sp. *E. coli* with its higher enzyme activity is more important in the deconjugation of bilirubin diglucuronide (Swidsinski and Lee, 2001).

Polymerase chain reaction (PCR) was used to amplify uidA (encoding *Escherichia coli [E. coli]* beta-glucuronidase) genes in different types of gallstones. PCR products were sequenced.Bacterial DNA sequences are usually present in mixed cholesterol (to 95% cholesterol content), brown pigment, and common bile duct, but rarely in pure cholesterol gallstones. The presence of bacterial beta-glucuronidase is also suggested. The role of bacteria and their products in the formation of mixed cholesterol gallstones, which comprise the majority of cholesterol gallstones, warrants further study.

Numerous investigators have proposed a role for bacteria in biliary lithogenesis. We hypothesized that bacterial DNA is present in gallstones, and that categorical differences exist between gallstone type and the frequency of bacterial sequences.

Bacterial 16S rRNA and *uidA* DNA sequences in *E. coli* were detected in all brown pigment, common bile duct, and mixed cholesterol gallstones (n = 14). In contrast, only one (14%) of seven pure cholesterol gallstones yielded a PCR product. Most (88%) mixed cholesterol gallstones yielded PCR amplification products from their central, as well as their outer, portions. Sequenced products possessed 88–98% identity to 16S rRNA genes of *E. coli* and *Pseudomonas*species (Lee *et.al*., 1999).

Bacterial infection is accepted as a precipitating factor in cholesterol gallstone formation, and recent studies have revealed the presence of *E. coli* species in the hepatobiliary system. We utilized the polymerase chain reaction (PCR) to establish the presence of bacterial DNA, including from *E. coli* species, in gallstones, bile juice,

and gallbladder mucosa from patients with gallstones (Lee, *et.al*., 2010).

Figure 2: Agarose gel electrophoresis presenting the PCR results obtained with *uidA* primers. Lane M, molecular mass marker (100 – 1500 bp range); lanes **12 ,4** *uidA* -positive *E. coli*; lanes 1,2,3,5,6,7,8,9,10,11 *uidA* - negative *E. coli* .

References

[1] Bej, A. K.; Di Cesare, J. L.; Haff, L. and Atlas, R. M. (1991a). Detection of Escherichia coli and *Shigella spp*. in water by using the polymerase chain reaction and genes probes for uid. *Appl . Environ. Microbiol.*, 57(4): 1013–1017.

[2] Bej, A. K.; Mc Carty, S. C. and Atlas, R. M.(1991b). Detection of coliform bacteria and Escherichia coli by multiplex Polymerase Chain Reaction: comparison with defined substrate and plating methods for water quality monitoring. Appl. Environ. Microbiol. 57(8): 2429– 2432.

[3] Choi, C.; Kwon, D. and Chae, C. (2001). Prevalence of the entero-aggregative *Escherichia coli* heat-stable enterotoxin 1 gene and its relationship with fimbria and toxin genes in *E. coli* isolated from diarrheic piglets. *J. Vet. Diagn.Invest.*, 13: 26-29.

[4] Collee, J.; Garald, G.; Fraser, F.; Andrew, G.; Marmion, M.; Barrie,P.; Simmon, S. and Anthong, N. (1996). Practical medical micro-biology.14th ed. Churchill Livingstone.Newyork., P: 131-150.

[5] Feng, P.; Lum, R. and Chang, G.W.(1991). Identification of uidA Gene Sequences in B-D-

[6] Glucuronidase-Negative Escherichia coli.*Appl. Environ. Microbial*, 57(1): 320-323.

[7] Frydendahl K.(2002). Prevalence of serogroups and virulence genes in *Escherichia coli* associated with postweaningdiarrhoea and edema disease in pigs and a comparison of diagnostic approaches. *Microbiol*., 85**:** 169-182.

[8] Holt, J.G.; Kreig, N.R.; Sneath, P.H.A.; Staley, J.T. and Williams, S.T. (1994). In Bergey's Manual of Determinative Bacteriology, 9th .ed. The Williams and Wilkins Co., Baltimore.

[9] Iqbal, S.; Robinson, J.; Deere, D.; Saunders, J. R.; Edwards, C.and Porter, J. (1997).Efficiency of the polymerase chain reaction amplification of the uid gene for detection of Escherichia coli in contaminated water.*Lett. Appl. Microbiol*., 24: 498–502.

[10] Jefferson, R. A.; Burgess, S. M. and Hirsh, D.(1986). F-glucuronidase from *Escherichia coli* as a gene-fusion marker. Proc. Natl. Acad. Sci. USA 83:8447-8451.

[11] Lasalde, C.; Huertas, A.; Rodrı´guez, R.; Toranzos, G. A. (2003). *Escherichia coli* populations and uidA gene variation in atropical rain forest soil. 103rd American Society for Microbiology (ASM) General Meeting. Washington, DC. pp: 18–22.

[12] Lee, D.K.; Tarr, P.I.; Haigh, W.G. and Lee, S.P. (1999).Bacterial DNA in mixed cholesterol gallstone. *Am. J .Gastroenterol*., 94 (12) : 3502-3506.

[13] Lee, J.W.; Lee, D.H.; Lee, J.I.; Jeong, S.; Kwon, K.S.; Kim, H.G.; Shin, Y.W.; Kim, Y.S.; Choi, M.S. and Song, S.Y.(2010). Identification of Helicobacter pylori in Gallstone, Bile, and Other Hepatobiliary Tissues of Patients with Cholecystitis .*Nat. Inst. Health*, 4(1):60-67.

[14] Martins, M. T., Rivera, I. G., Clark, D. L., Stewart, M. H., Wolfe,R.L. and Olson, B. H. (1993). Distribution of uidA gene sequences in *Escherichia coli* isolates in water sources and comparison with the expression of b-glucuronidase activity in 4-methylumbelliferyl- b-D-glucuronide media. *Appl. Environ. Microbiol.*, 59(7): 2271–2276.

[15] Mc Daniels, A. E.; Rice, E. W.; Reyes, A. L.; Johnson, C. H.; Haugland, R. A. and Stelma, G. N. (1996).Confirmational identification of *Escherichia coli*, a comparison of genotypic and phenotypic assays for glutamate decarboxylase and b-D-glucuronidase. *Appl. Environ. Microbiol.*, 62(9):3350–3354.

[16] Monday,S. R.; Whittam, T. S. and Feng, P. C. H.(2001). Genetic and evolutionary analysis of mutations in the gusA gene that cause the absence of b-Glucuronidase activity in *Escherichia coli* O157:H7. *J. Infect. Dis*., 184: 918–921.

[17] Muller, D.; Greune, L.; Heusipp, G.; Karch, H.; Fruth, A.; Tschape, H. and Schmidt A.M.(2007).Identification of unconventional intestinal pathogenic *E.coli* isolates expressing intermediate virulence facter profiles by using novel single-step multiplex PCR. *Appl. and Env.Micro*., 73(10):3380-3390.

[18] Nagy, B.andFekete, P.Z. (1999). Enterotoxigenic *Escherichia coli* (ETEC) in farm animals. Vet. Res. 30**:** 259-284.

[19] Nataro, J.P. and Kaper, J.B.(1998). Diarrheagenic*Escherichia coli*.Clin.Microbiol. Rev., 11: 142-201.

[20] Osek, J.(2003). Identification of the astA gene in enterotoxigenic *Escherichia coli* responsible for diarrhea in pigs. *Bull. Vet. Inst. Pulawy., 47*: 9-15.

[21] Osek, J.(1999). Prevalence of virulence factors of *Escherichia coli* strains isolated from diarrheic and healthy piglets after weaning. Vet. *Microbiol* . 68**:** 209-217.

[22] Rompre, A.; Servais, P.; Baudart, J.; de-Roubin, M. and Laurent, P. (2002) . Detection and enumeration of coliforms in drinking water: current methods and emerging approaches. *J. Microbiol. Meth.*, 49: 31–54.

[23] Scaletsky, I.C.A.; Silva, M.L.M. and Trabulsi, L.R. (1984). Distinctive patterns of adherence of enteropathogenic*Escherichia coli* to HeLa cells. Infect. Immun.45**:** 534-536.

[24] Spitz, J.; Yuhan, R. and Koutsouris, A. (1995).Enteropathogenic*Eschericha coli* adherence to intestinal epithelinal monolayers diminishes barrier function. *Am.J.Physiol*., 268:374-9.

[25] Swidsinski, A. ; and Lee S.P. (2001) .The role of bacteria in gallstone pathogenesis. Frontiers in Bioscience. 6: 93-103.

[26] Tsai, Y.; Palmer, C. J. and Sangermano, L. R. (1993). Detection of Escherichia coli in sewage sludge by polymerase chain reaction. *Appl. Environ. Microbiol*., 59(2): 353–357.

[27] Yamamoto,T. and Echeverria,P.(1996).Detection of the enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 gene sequences in enterotoxigenic*E. coli* strainspathogenic for humans. *Infect. Immun.*, 64: 1441-1445.

[28] Yamamoto, T.; Wakisaka, N.; Sato, F. and Kato, A.(1997).Comparison of the nucleotide sequence of enteroaggregative*Escherichia coli* heat- stable enterotoxin 1 genes among diarrhea-associated *Escherichia coli*. *FEMS Microbiol.Lett.*, 147:89-95.

[29] Yamamoto, T. and Nakazawa, M.(1997). Detection and sequences of the enteroaggregative*Escherichia coli* heat-stable enterotoxin 1 gene in enterotoxigenic*E. coli* strains isolated from piglets and calves with diarrhea. *J. Clin. Microbiol*., 35: 223-227