An alternative approach for evaluating the phenotypic virulence factors of pathogenic *Escherichia coli*

Kamelia M. Osman, Ashgan M. Hessain, Usama H. Abo-shama, Zeinab M. Girh, Saleh A. Kabli, Hassan A. Hemeeg, Ihab M. Moussa

*Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt*
*Department of Health Science, College of Applied Studies and Community Service, King Saud University, Saudi Arabia*
*Department of Poultry Diseases, National Research Center, Dokki, Egypt*
*Department of Biology, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah, Saudi Arabia*
*Department of Medical Technology/Microbiology, College of Applied Medical Science, Taibah University, Madinah, Saudi Arabia*
*Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia*
*Microbiology & Immunity Department, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt*

Abstract

*Escherichia coli* is a recognized zoonotic food-borne pathogen; however, the use of polymerase chain reaction (PCR) in underdeveloped countries to differentiate pathogenic from non-pathogenic *E. coli* is a problematic issue. Our goal was to assess the phenotypic virulence markers motility, hemolysin, Congo red binding, Embryo Lethality assay (ELA) and serum resistance, as well as antibiotic susceptibility using disc diffusion method to 23 antibiotics. Results exhibited 100% motility and Congo red binding, 97.1% for hemolysin production and 90.2% in the ELA. As a result, we were able to hypothetically conclude that the aforementioned virulence markers are plain, straightforward, economical, rapid, more dynamic, uncomplicated methodology, duplicatable and cost next to nothing when compared to the molecular PCR. Their implementation in a diagnostic microbiology laboratory for vetting is a rewarding task in the underdeveloped countries. It augments endeavors to minimize the use of PCR in our investigations especially during epidemiological and outbreak investigations of PEC.

Article info

Article history:
Received 14 February 2017
Revised 13 April 2017
Accepted 1 May 2017
Available online 3 May 2017

Keywords:
Pathogenic *E. coli*
Motility
Haemolysin
Congo red
Embryo lethality assay
Serum resistance

1. Introduction

*Escherichia coli* has been differentiated into more than 50,000 different serotypes, of which several have the capability to produce disease through their pathogenic potentiality (*EFSA*, 2014; *CDC*, 2015). There are several methods for detecting virulence to discriminate among pathogenic and non-pathogenic *E. coli* serovars which comprise classical phenotypic cultural vetting procedures and molecular techniques. However, many laboratories throughout the world, when we specially refer to the underdeveloped countries, lack the ability or the skilful manpower needed to evolve molecular procedures to detect the pathogenic *E. coli* isolates, maintenance and repair of the sophisticated equipments that are used in the molecular assays. It is therefore essential that other, cheaper and non-sophisticated scanning procedures are integrated into standard medical métier and diagnostic laboratories in the Third World.

Therefore, our goal was to assess the phenotypic factors (motility, haemolysin, Congo red agar, ELA, serum resistance and PCR) as predictors of virulence and as a sensitive and specific tool for the pathogenicity of *E. coli* isolated in the diagnostic microbiological laboratories of the underdeveloped countries to differentiate between pathogenic and non-pathogenic *E. coli* isolated from different sources [poultry house environment (air, labor hands, litter, water), poultry, cattle, sheep and goat] in the failing of molecular biology potentiality.
2. Materials and methods

The 448 strains we studied were previously isolated and molecularly identified from different sources (Osman et al., 2012a, 2012b; Osman et al., 2013). After being re-confirmed as E. coli, the 448 purified isolates were tested for their pathogenicity using classical tests for E. coli pathogenicity as previously described by Osman et al. (2012a, 2012b) which included motility, hemolytic activity, Congo red uptake (CR), Embryo Lethality Assay (ELA), and assessment of serum resistance (SR).

3. Results and discussion

The results of the phenotypic virulence markers recorded that 348 E. coli isolates were found to be 100% motile, 97% were hemolytic. 95% were Congo red positive, Embryo lethality at 13th day post-inoculation, ranged from 10.0 to 100% with a mean of 36% and 62% of the E. coli isolates were highly sensitive to serum resistance as shown in Table 1.

E. coli is a highly adaptable microorganism that has evolved sophisticated means of variable phenotype virulence tests, motility and hemolysis are sometimes tested in conjunction with complement resistance and embryo lethality tests to differentiate pathogenic E. coli (Dziva and Stevens, 2008). All of our 448 E. coli isolates were found to be motile mediated by the flagella of E. coli, which is one of the virulence factors (Lane et al., 2005; Chelsea et al., 2007; Tonu et al., 2011) for pathogenicity of E. coli (Kao et al., 2014). The other phenotypic virulence markers are: (i) the characteristic CR binding affinity (Al-Saiedi and Al-Mayah, 2014; Yadav et al., 2014), (ii) the ELA was found to have the potentiality to differentiate between highly virulent, moderately virulent, and avirulent isolates of avian E. coli (Wooley et al., 2000; Gibbs and Woolley, 2003; Gibbs et al., 2003, 2004; Oh et al., 2012), (iii) the capacity to counteract the germicidal action of serum (serum resistance), and thus continue to live in the bloodstream, represents another essential pathogenic phenomenon for pathogenic E. coli strains (Falkenhagen et al., 1991; Jacobson et al., 1992; Allan et al., 1993), (iv) hemolysis production has been implicated as an emerging and one of the most important virulence factors (Fatima et al., 2012). The present study showed that 97% of our E. coli isolates were able to produce hemolysis, a phenotypic virulence phenomenon demonstrated in a number of pathogens such as streptococcal and staphylococcal species, E. coli, Serpulina hyodysenteriae, Mycobacterium tuberculosis, Trypanosoma cruzi, and Listeria monocytogenes (Braun and Focareta, 1991; Andrews and Portnoy, 1994; Bhakdi et al., 1996; Morgan et al., 1996; Beutin, 1999; Quave et al., 2015). The cytolytic protein toxin, also known as cytotoxic necrotizing factor, hemolysin, secreted by the majority of pathogenic E. coli strains produced cell-associated lysin on blood agar plates seen as a clear zone of lysis (Smith, 1963; Shobrak and Abo-Amer, 2014). The importance of the hemolysin criteria, especially α-hemolysin, comes from the fact that it is strongly proinflammatory leading to secretion of IL-6 and chemoattractins, which sets pace for the pathogenesis of renal disease (Ranjan et al., 2010; Garcia et al., 2013). Clinical studies have indicated that the virulence factors of E. coli like production of hemolysin and the capacity to counteract the germicidal action of serum play a role in the pathogenesis (Fatima et al., 2012; Rizvi et al., 2013).

4. Conclusion

We were able to hypothetically conclude that the aforementioned virulence markers are plain, straightforward, economical, rapid, more dynamic, uncomplicated methodology, duplicatable and cost next to nothing when compared to the molecular PCR (Table 1). Their implementation in a diagnostic microbiology laboratory for vetting is a rewarding task in the underdeveloped countries. It augments endeavors to minimize the use of PCR in our investigations especially during epidemiological and outbreak investigations of PEC.

Acknowledgment

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project no.: RG-162.

References

Allan, B.J., Iven den Hurk, J.V., Potter, A.A., 1993. Characterization of Escherichia coli isolated from cases of avian colibacillosis. Can. J. Vet. Res. 57, 146–151.
Al-Saiedi, R.R., Al-Mayah, A.A.S., 2014. Pathogenicity testing of several APEC isolates obtained from naturally infected broiler birds reared in Basrah. Int. J. Poult. Sci. 13, 374–378.
Andrews, N.W., Portnoy, D.A., 1994. Cytolysins from intracellular pathogens. Trends Microbiol. 2, 261–263.
Beutin, L., 1999. Escherichia coli as a pathogen in dogs and cats. Vet. Res. BMC 30, 285–298.
Bhakdi, S., Bayley, H., Valeva, A., Walev, M., Palmer, M., 1996. Staphylococcal alpha-toxin, streptolysin-O, and Escherichia coli hemolysin: prototypes of pore-forming bacterial cytolytins. Arch. Microbiol. 165, 73–79.
Braun, V., Focareta, T.M., 1991. Pore-forming bacterial protein hemolysins (cytolysins). Crit. Rev. Microbiol. 18, 115–158.
CDC, 2015. Centers for Disease Control and Prevention Escherichia coli (E. coli) 1600 Clifton Road Atlanta, GA 30329-4027, USA, 800-CDC-INFO (800–232-4636), TTY: 888-232-6148 Email CDC-INFO April 16, 2015.
Chelsea, L.M., Simms, A.N., Mobley, H.L.T., 2007. Complex interplay between type 1 fimbrial expression and flagellum-mediated motility of uropathogenic Escherichia coli. J. Bacteriol. 189, 5523–5533.
Dziva, F., Stevens, M.P., 2008. Colibacillosis in poultry: unraveling the molecular basis of virulence of avian pathogenic Escherichia coli in their natural hosts. Avian Pathol. 37, 355–356.
EFSA, 2014. European Food Safety Authority. Escherichia coli (E. coli) 2014. Via Carlo Magno 1A, 43126 Parma, Italy. <www.efsa.europa.eu>. http://dx.doi.org/10.2805/62059. ISBN: 978-92-9199-612-4.
Falkenhagen, U., Zingler, G., Naumann, C., 1991. Serum resistance in different serotypes of Escherichia coli. Zentralbl. Bakteriol. 275, 216–222.
Fatima, N., Agrawal, M., Shukla, I., Khan, P.A., 2012. Characterization of uropathogenic E. coli in relation to virulence factors. Sci. Rep. 1, 342. doi: 10.4172.
Garcia, T.A., Ventura, C.L., Smith, M.A., Merrell, D.S., O’Brien, A.D., 2013. Cytotoxic necrotizing factor 1 and hemolysin from uropathogenic Escherichia coli elicit different host responses in the murine bladder. Infect. Immun. 81, 99–109.
Gibbs, P.S., Maurer, J.J., Nolan, L.K., Wooley, R.E., 2003. Prediction of chicken embryo lethality with the avian Escherichia coli traits complement resistance, colicin V production, and presence of the increased serum survival gene cluster (iss). Avian Dis. 47, 370–379.
Gibbs, P.S., Petermann, S.R., Wooley, R.E., 2004. Comparison of several challenge models for studies in avian colibacillosis. Avian Dis. 48, 751–758.
Gibbs, P.S., Wooley, R.E., 2003. Comparison of the intravenous chicken challenge method with the embryo lethality assay for studies in avian colibacillosis. Avian Dis. 47, 672–680.
Jacobson, S.H., Östenson, C.G., Tullus, K., Brauner, A., 1992. Serum resistance in Escherichia coli strains causing acute pyelonephritis and bacteraemia. Acta Pathol. Microbiol. Immunol. Scand. 100, 147–153.
Kao, C.Y., Lin, W.H., Tseng, C.C., Wu, A.B., Wang, M.C., Wu, J.J., 2014. The complex interplay among bacterial motility and virulence factors in different Escherichia coli infections. Eur. J. Clin. Microbiol. Infect. Dis. 33, 2157–2162.
Lane, M.C., Lockatell, V., Monterosso, G., Lamphier, D., Weinert, J., Hebel, J.R., Johnson, D.E., Mobley, H.L.T., 2005. Role of motility in the colonization of uropathogenic Escherichia coli in the urinary tract. Infect. Immun. 73, 7644–7656.
Morgan, P.J., Andrew, P.W., Mitchell, T.J., 1996. Thiol-activated cytolysins. Rev. Med. Microbiol. 7, 221–229.
Oh, J.Y., Kang, M.S., Yoon, H., Choi, H.W., An, B.K., Shin, E.G., Kim, Y.J., Kim, M.J., Kwon, J.H., Kwon, Y.K., 2012. The embryo lethality of Escherichia coli isolates and its relationship to the presence of virulence-associated genes. Poult. Sci. 91, 370–375.
Osman, K.M., Mustafa, A.M., Aly, M.A.K., AbdElhamed, G.S., 2012a. Serotypes, virulence genes, and intimin types of shiga toxin-producing Escherichia coli and enteropathogenic Escherichia coli isolated from mastitic milk relevant to human health in Egypt. Vector Borne Zoonotic Dis. 12, 297–305.
Osman, K.M., Mustafa, A.M., Elhariri, M., AbdElhamed, G.S., 2012b. Identification of serotypes and virulence markers of Escherichia coli isolated from human stool and urine samples in Egypt. Ind. J. Med. Microbiol. 30, 308–313.
Ranjan, K.P., Ranjan, N., Chakraborty, A., Arora, D.R., 2010. An approach to uropathogenic Escherichia coli in urinary tract infections. J. Lab. Phys. 2, 70–73.
Rizvi, M., Kumar, S., Khan, F., Sultan, A., Malik, A., Tahira, F., 2013. Killing of Ipha-hemolytic and non-hemolytic Escherichia coli strains from paediatric patients in human serum and polymorphonuclear leucocytes. Int. J. Curr. Microbiol. Appl. Sci. 2, 636–646.
Shobrak, M.Y., Abo-Amer, A.E., 2014. Role of wild birds as carriers of multi-drug resistant Escherichia coli and Escherichia vulneris. Braz. J. Microbiol. 45, 1199–1209.
Smith, H.W., 1963. The hemolysins of Escherichia coli. J. Pathol. Bacteriol. 85, 197–211.
Tonu, N.S., Sufian, M.A., Sarker, S., Kamal, M.M., Rahman, M.H., Hossain, M.M., 2011. Pathological study on colibacillosis in chickens and detection of Escherichia coli by PCR. Bangl. J. Vet. Med. 9, 17–25.
Wooley, R.E., Gibbs, P.S., Brown, T.P., Mauer, J.J., 2000. Chicken embryo lethality assay for determining the virulence of avian Escherichia coli isolates. Avian Dis. 44, 318–324.
Yadav, V., Joshi, R.K., Joshi, N., Diwakar, R.P., 2014. Congo red binding and plasmid profile of E. coli isolates of poultry origin. J. Anim. Health Prod. 2, 31–32.