Saudi Journal of Biological Sciences 25 (2018) 195–197

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

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

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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

ABSTRACT

*Escherichia coli* is a recognized zoonotic food-borne pathogen; however, the use of polymerase chain reaction (PCR) in the underdeveloped countries to differentiate pathogenic from non-pathogenic *E. coli* is a problematic issue. Our grail was to assess the phenotypic virulence markers motility, hemolysin, congo red agar, embryo lethality assay and serum resistance for pathogenic *E. coli* (PEC) correlated to PCR tests which is currently used world-wide to evaluate the PEC. The 448 strains of *Escherichia coli* that were isolated from different sources, were characterized for phenotypic virulence factors such as 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.


## 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, hemolysin, 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.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2017.05.002
1319-562X/

**Table 1**
Comparison of the pathogenic *E. coli* phenotypic virulence markers.

| | Motility | Congo Red | Hemolysin | Embryo lethality assay | Serum resistance assay | PCR |
|---|---|---|---|---|---|---|
| Cost | Very Low | Very Low | Very Low | Low | Very Low | High |
| Ingredients | Very Low | Very Low | Very Low | Low | Very Low | High |
| Equipment | Very Low | Very Low | Very Low | Low | Very Low | High |
| Maintenance | Very Low | Very Low | Very Low | Low | Very Low | High |
| Labor | Very Low | Very Low | Very Low | Low | Very Low | High |
| Sample | Very Low | Very Low | Very Low | Low | Very Low | High |
| Speed | 24 h | 24–48 h | 24–48 h | 7 days | 24 h | ≤24 h |
| Automation | Poor | Poor | Poor | Poor | Poor | Good |
| Sensitivity | Very good | Very good | Very good | Very good | Very good | Excellent |
| Specificity | Very good | Very good | Very good | Very good | Very good | Excellent |

## 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 Wooley, 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) hemolysin 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 chemotaxins, 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.

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