



Influence of Plasmids on the Pathogenicity and Virulence of *Escherichia coli* Strains of Human and Animal Origin

Renu Goel¹

¹Department of Zoology, P.P.N. PG College, Kanpur.

Escherichia coli usually considered to be an opportunistic pathogen which constitutes a large proportion of the normal intestinal flora of men and animals. This organism can, however, contaminate, colonize and subsequently cause infection of extra intestinal sites and is a major cause of septicaemia, peritonitis, abscesses, meningitis and urogenital tract infections in men and animals. The source of these *E. coli* pathogens in most cases is believed to be the host's own intestinal flora. Because of its clinical significance, *E. coli* has been the subject of numerous investigations in an attempt to define those virulence factors which allow it to initiate and sustain infection. *E. coli* is recognized as a gram-negative rod shaped bacterium, which ferments lactose and produces characteristic colonies on certain differential bacteriologic media such as MacConkey's agar. Several schemes have been developed to characterize isolates of *E. coli* and to aid in the identification of pathogenic strains: serotyping, biotyping, phage-typing, colicin-typing and testing for virulence factors.

Serotyping is the most widely used of these methods and an international scheme has been established on the basis of O (Cell wall) K (capsular) and H (flagellar) antigens. Approximately, 160 O, 100 K and 60 H antigens have been identified in this scheme (Qrskov, 1984).

Biotype based on the patterns of reaction in selected biochemical tests, can be related to serotypes but the absence of universal biotyping scheme severely limits this approach, the method is not widely used for characterizing isolates of pathogenic *E. coli*. Phage typing is carried out by determining the susceptibility of isolates to a standard set of bacterial phages. Colicin typing involves detecting the production of colicin (s) by an isolate, then determining the effect of the colicin (s) on a standard set of indicator strains of *E. coli*. Neither of these schemes is in common use of identifying pathogenic isolates of *E. coli*.

Recent development have made it possible to determine whether certain isolates possess specific properties responsible for pathogenicity. Advances have been mostly in association with pathogenicity in the gut and have contributed to our ability to differentiate *E. coli* that are normal gut flora from *E. coli* that are involved in enteric diseases. (C. Kang et al., 2018)

Several structures and products of *E. coli* have either a demonstrable or a potential role in virulence in the gut and other tissues. These structures include flagella, capsule, cell wall and pili (fimbriae), the products include colicins, enterotoxins, cytotoxins and haemolysins. (M.A. Smith, et al. 2015)

Bacterial plasmids are extrachromosomal circular, double standard DNA elements of bacteria that constitutes a reasonable stable but dispensable gene pool. Each plasmid autonomously replicate and controls its own copy number. Under normal circumstances that are not essential for the successful growth and metabolism of their host bacterium (Gahamanyi et al., 2020). Often, however, these genetic elements carry genes for supplementary activity that allows their host to better survive in adverse environment or permit their host to compete more successfully with micro-organism of the same or different species in the intestinal tract. (Barnier et al., 2021)

Genetic variation is also important in the evolution of lower organisms such as bacteria, and here too it arises from mutations. Bacteria have only one chromosome, however, so that different alleles of a gene are not normally present within single cell. The reshuffling of a bacterial genes, therefore, ordinarily requires the introduction into a bacterium of DNA carrying an allele that originated in a different cell. One mechanism accomplishing this intrabacterial transfer of genes in nature is transduction (Denis et al, 2019). Certain virus that can infect bacterial cells pick up fragments of the bacterial DNA and carry this DNA to other cells in the course of a later infection. In another process, known as “transformation” DNA released by cell death or other natural processes, simply enters a new cell from the environment by penetrating the cell wall and membrane. A third mechanism, ‘conjugation’ involves certain of the self replicating circular segments of DNA called plasmids, which can be transferred between bacterial cells that are in direct physical contact with each other. Whether the genetic information is introduced into a bacterial cell by transduction, transformation or conjugation, it must be incorporated into the new host’s hereditary apparatus if it is to be propagated as part of that apparatus when the cell divides.

The genetic units can mediate conjugal DNA transfer, resistance to many antibiotics and divalent cations degradation of organic compounds (such as naphthalene, hexachlorophene) and some key diagnostic biochemical properties (Such as urease or hydrogen sulphide production or lactose utilization). Plasmids are uniquely responsible for several diseases of plants and animals. (Tam et al., 2019)

Plasmids generally range in size from about 5000 to 4,50,5000 nucleotide base pairs, i.e. each plasmid can potentially encode from 5 to more than 400 average sized accessory proteins. Moreover, a single bacterial strain will often harbour several plasmid types simultaneously.

Recently, it has become increasingly evident that plasmid code certain properties that are essential to the virulence of many different pathogenic bacteria. These plasmids are medically important because of their transferable nature which allow them to spread in non-pathogenic bacteria and turn them to pathogenic. (Borg et al., 2020)

In general, the virulence of a strain of a pathogenic species is determined by two factors its invasiveness or ability to proliferate in the body of host and its toxigenicity or ability to produce chemical substances or toxins that damage the tissue of the hosts. But the research in past

decades about bacterial pathogenicity have been reached at this conclusion that some strains are non-invasive and non-enterotoxigenic, yet they are pathogenic and virulent. It means some other factors are also involved in bacterial pathogenicity. (Goel R., 2019)

Some plasmid mediated properties of *E. coli* eg. The production of colicin (Ansari and Yadava, 1981), haemolysin (Ahmad and Yadava, 1980), haemagglutination (Minschew et al., 1978), drug resistance (Yadava et al., 1982) and serum resistance (Yadava et al., 1982) have been reported to be closely associated with virulence of *E. coli* strains.

Multiple antibiotic resistance due to plasmid was discovered in Japan 27 years ago (Akiba et al., 1960). Since then an impressive volume of epidemiological data has accumulated which demonstrates unequivocally that the wide spread and increasing occurrence of such R-plasmids or so-called R-factors present in bacteria progressively reduce or at least complicate effective antibiotic treatment of human and animal bacterial infections. During the last five years the problem of multiple drug resistance has occupied the mind of research workers concerning the veterinary, medical and biological specialities.

The clones of resistant organism may predominate in hospital infections as a result of antibiotic pressure and the plasmid may also provide additional virulence factors to pathogen (Reynard and Beck, 1976). A few wild type strains of *E. coli* has been shown to acquire serum resistance following acquisition of certain R-plasmids (Moll et al., 1980).

The 1964, Mark Richmond and Eric Johnson of National Institute of Medical Research in England showed that the Staphylococci responsible for an outbreak of post-surgical 'suture line' infections carried on a plasmid enabling not only to destroy penicillin but also to grow in the mercury based disinfectant used to sterilize the sutures. In the laboratory at the Public Health Research Institute of the city of New York, it was found that staphylococcus plasmids carried genes for resistance to penicillin and mercury compounds and also to a variety of other heavy metal compounds normally lethal to staphylococci. Various heavy metal resistance genes have also been found on some *E. coli* R-plasmids. (M.J. Lee et al. 2015)

Apart from the rather special nature of many of their functions, plasmid genes are not basically different from other genes.

One of the important plasmid encoded property is the ability to produce haemolysin which is released extracellularly and lyse RBCs of several species of animals and human. A high proportion of extra intestinal *E. coli* strains and those isolated from other sources of infections also produce haemolysins, and it, therefore, has been proposed to be a virulent factor associated with bacterial pathogenicity (Minschew et al., 1978).

Certain strains of *E. coli* cause a disease in human beings resembles bacillary dysentery. A common characteristic of these strains is their ability to invade the epithelial cells of the colon. Mutant of virulent strains producing dysentery like disease which fail to penetrate epithelial cells also fail to cause disease and behave differently from pathogenic *E. coli* in animal models (Labrec et al., 1964).

Bacterial surface structures are also related to the properties of virulence. Although these antigens can be associated with invasiveness, resistance to phagocytosis and intestinal colonization. No single factor or antigen determines pathogenicity. Among enterobacteriaceae there are two categories of surface antigens, those composed of polysaccharide and those composed of proteins.

The polysaccharide antigens can be divided into lipopolysaccharide (O-antigens) and capsular polysaccharide (K-antigens). The lipopolysaccharide have three regions: (i) Lipid-A, (ii) Core oligosaccharide and (iii) O-specific polysaccharide. Lipid-A is responsible for the toxic properties of the lipopolysaccharide and is probably similar in the different enterobacteriaceae (E. Esteve et al. 2018). The structure of core polysaccharide (region II) present in enterobacteria varies eg. the same structure has been detected in all Salmonella but four different ones have been found in E. coli.

The O-specific polysaccharide is characteristic of the smooth (S) form of enterobacteriaceae. This polysaccharide is the chemical basis of the O-antigen specificity of gram-negative bacteria. The various O-antigens are specified by the different sugars found in this O-specific side chain. Mutation affecting the synthesis of O-specific side chain may result in rough (R) variants that give R-specificity to the core polysaccharide. Since R-mutants are easily phagocytised and more sensitive to bactericidal activities, they are less pathogenic and less virulent than S-forms (Hanson, 1973).

Further research in the area of association of virulence factors of bacterial surface, i.e. pili or fimbriae or haemagglutinins is needed. Pili or fimbriae are non-flagellar filamentous surface antigens are often associated with the E. coli strains pathogenic for different animal species are antigenically distinct. But they share a number of chemical and biological properties. These pili are composed of hydrophobic proteins (Brinton, 1965). Besides bacterial binding due to this general stickiness specific attachment to certain host and tissues occur (Gibbon and Houte, 1975) and is thought to be a virulence factor for bacterial colonizing or causing infection of mucous surfaces. Bacterial adhesions have been classified according to the agglutination patterns resulting when bacteria bind to erythrocyte from various species.

E. coli strains belonging to various infections of mammals have been investigated in the present study in order to elucidate the possible role played by plasmids in the pathogenesis of these infections. An attempt has also been made to transfer the virulent haemolytic factor to the non-haemolytic recipient strains of E. coli in order to ascertain the significance of plasmid in the transfer of pathogenic character to non-pathogenic bacterial strains. Various E. coli strains were also checked for their haemagglutination (adhering) activity. The transfer of this character to non-haemagglutinating strains by conjugation to assess the presence of adhesions (colonization factor) on the bacterial surface, and its effect on the pathogenicity of recipient strain has been reported.

In the light of the above facts, the present problem was undertaken to study in detail the effect of various other plasmids on the pathogenicity and virulence of gram negative bacteria, which may lead to a better approach of chemotherapy and control of bacterial infections.

References

- Ahmad, S. and Yadava, J.N.S. (1980). Haemolysin production among *E. coli* strains isolated from clinical cases of man and animal. *Indian Vet. Med. J.* 4 (4): 149-154.
- Akiba, T.; Koyama, K; Ishiki, Y.; Kimura, S. and Fukushima, T. (1960). On the mechanism of the development of multiple drug resistance clones of *Shigella*. *Jap. J. Microbiol.* 4: 219.
- Ansari, M.Q. and Yadava, J.N.S. (1981). Note on colicinogeny and lysogeny among clinical isolates of *E. coli* from human and animal sources. *Indian J. Anim. Sci.* 51 (7): 664-666.
- Barnier, J.P., Euphrasie, D. Join-Lambest O, Audry M. Schonherr-Hellec- S., Schmitt T. Bourdoulous S, Coureuil M., Nassif, ElBehiM 2021. Type-IV pilus retraction enables sustained bacteremia and plays a key role in the outcome of meningococcal sepsis in a humanized mouse model *PLOS Pathog*17: c 1009299.
- Borg, M.A.; Camilleri, L. What is driving the Epidemiology of Methicillin-Resistant *Staphylococcus aureus* infection in Europe? *Microbiol. Drug Resist.* 2020, 27, 889- 894.
- Brinton, C.C. (1965). The structure, function, synthesis and genetic control of bacterial pili and a molecular model for DNA and RNA transport in gram negative bacteria. *Trans. N.Y. Acad. Sci.* 27: 1003-1054.
- C. Kang, J. Kim, D.W. Park et al. (2018), “Clinical practice guidelines for the antibiotic treatment of community acquired UTI”. *Infection and Chemotherapy*, Vol. 50, No. 1, pp. 67-100.
- Denis K., Le BrisM, Le Guennec L, Barnier JP, Faure C, Gouge A, Bouzinba-Segard H, Jamet A, Euphrasie D, Dural B. Barois N. Pellisier, P. Morand PC, Coureuil M; Lafont F; Join – Lambert O; Nassif X; Bourdoulous S. (2019). Targetting type IV pili as an antivirulence strategy against invasive meningococcal disease. *Nat. Microbiol* 4: 972-984.
- E. Esteve – Palau, S. Grau, S. Herrera et al. “Impact of an antimicrobial Stewardship program on UTIs caused by extended spectrum beta lactamase producing *Escherichia coli*” *Revista Espanola De Quimioterapia* (2018).
- Gahamanyi N. Mboera LEG, Matee MI, Mutangana D., Komba EVG. Prevalence, risk factors and antimicrobial resistance profiles of thesmophilic campylobacter species in humans and animals in sub-saharanAfrica: a systematic review. *Int J. Microbiol.* (2020) 2020: 1-12, doi: 10.1155/2020/2092478.
- Gibbons, R.J. and Houte, J. Van (1975). Bacterial adhesion in oral microbial ecology. *Ann. Rev. Microbiol.* 29: 19-44.
- Goel, R. (2019). Effect of drug resistance on the pathogenicity and virulence of *Escherichia coli* PB-176. *Trends in Biosciences* 12(19): 1266-1269.
- Hanson, L.A. (1973). Host parasite relationship in UTI. *J. Infect. Dis.* 127 (6): 726- 730.
- Labrec, E.H.; Schneider, H.; Magnani, T.H. and Formal, S.B. (1964). Epithelial cell penetration as an essential step in the pathogenesis of bacillary dysentery. *J. Bacteriol.* 88 (5): 1503-1518.

- M.A. Smith, R.A. Weingarten, L.M. Ruso, C.L. Ventura, A.D.O' Brien. Antibodies against hemolysin and cytotoxic necrotizing factor type 1 (CNFI) reduce bladder inflammation in a mouse model of UTI infection with toxigenic uropathogenic *Escherichia coli*. *Infect. Immun.*, 83(4) (2015) pp. 1661-1673.
- M.J. Lee; M. Kim; N.H. Kim et al., Why is asymptomatic bacteriuria overtreated? A tertiary care institutional survey of resident physicians, "BMC Infectious Diseases, Vol. 15, p. 289 (2015).
- Minsheu, B.H.; Jorgensen, J.; Counts, G.W. and Falkow, S. (1978). Association of haemolysin production, haemagglutination of human erythrocytes and virulence for chicken embryos of extra-intestinal *E. coli* isolates. *Infect. Immun.* 20: 50-54.
- Minsheu, B.H.; Jorgensen, J.; Counts, G.W. and Falkow, S. (1978). Association of haemolysin production, haemagglutination embryos of extra intestinal *E. coli*. Isolates. *Infect Immun.* 20: 50-54.
- Moll, A.; Manning, P.A. and Timmis, K.N. (1980). Plasmid determined resistance to serum bactericidal activity: a major outer membrane protein, the tract gene product, is responsible for plasmid specified serum resistance in *E. coli*. *Infect. Immun.* 28 (2): 359-367.
- Qrskov, F. (1984). Genus *Escherichia*. In *Bergey's manual of systematic bacteriology* vol. 1, ed. NR, Kreig and J.G. Holt, Baltimore, Williams and Wilkins.
- Reynard, A.M. and Beck, M.E. (1976). Plasmid mediated resistance to the bactericidal effect of normal rabbit serum. *Infect. Immun.* 14 (3): 848-850.
- Tam, K.; Torres, V.J. *Staphylococcus aureus* secreted Toxins and extracellular enzymes, *Microbiol. Sectr*, 2019, 7-16.
- Yadava, J.N.S.; Goel, R. and Ansari, M.Q. (1982). Autotransferable R-plasmids in *E. coli* strains and their transfer to *S. typhimumin*, *Indian, Vet. Med. J.* 6 (4): 242-248.
- Yadava, J.N.S.; Goel, R. and Ansari, M.Q. (1982). Colorimetric serum resistance assay of drug resistant *E. coli* strains. *Curr. Sci.* 51 (21): 1041-1042.