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RESEARCH ARTICLE

GENOTYPIC CHARACTERIZATION OF TOXINS AND IRON ACQUISITION AGENT AS VIRULENCE FACTOR OF UROPATHOGENIC *ESCHERICHIA COLI.*

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Abstract

Escherichia coli called as UPEC possessing specific urovirulence factors, helps in adhesion, survival of the bacteria and leading to the infection in the urinary tract by surpassing the host immune response. Certain virulence factors are responsible for survival of the bacteria which is needed for long term persistence of the bacteria to cause recurrent infections. Aerobactin toxin helps in scavenging the iron from the host, while hemolysin A and cytotoxic necrotizing factor suppresses the inflammatory process and causes tissue damage. The aim of this study is to determine the presence of these fitness and virulence factor genes in the *E. coli* isolated from urine.

Introduction:-

Escherichia coli, a normal inhabitant of the human intestine becomes pathogenic in extraintestinal sites by acquisition of virulence factors induces urinary tract infection, neonatal meningitis, peritonitis and bacteriemia. Invasive toxins like α-hemolysin (hlyA), cytotoxic necrotizing factor (cnf) and an iron chelating agent - aerobactin (aer) are certain characteristics of UPEC [(1)]. **hlyA** and **cnf** operons encodes for exotoxins which are membrane active and stimulates PMN's cytoxicity respectively causing tissue damage and dysfunction of local immune responses. CNF toxin are mostly coproduced with alpha hemolysin, enhances the adherence to urothelial cells. The battle wages will always be present between the pathogens and iron limited host. Free iron (Ferric, Fe^{3+}cations is essential for the survival of pathogenic organism in its host. This iron-limited host cell environment is favoured to UPEC by an iron chelator - aerobactin which scavenges the ferric ions from the parasitized host [(2)].

Alpha hemolysin (HlyA) and its action in UTI:

It's a 107 kDa a membrane active protein induces hemolysis by creating 2nm wide pores in the erythrocyte membrane by increasing the permeability and swelling of cells leading to ruputure of RBCs which can be demonstrated invitro using Blood agar showing beta hemolysis. It's a Gly-rich nanopeptide protein repeated in tandem between 9 and 42 times which is named as RTX (Repeat in ToXin)[(3)].

hlyA binds to the hydrophobic surface of the bilayer membrane at neutral pH and lytic action on cell membrane requires calcium ion. *E.coli* uses endogeneous amplification, PX channel receptor especially PX 1, PX7 activation induces hemolysis in addition to that pannexin1, a pore forming protein in macrophages is a recently identified protein which increases the permeability for hemolysis[(4)].

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Cytotoxic necrotizing factor type 1 (CNF1) and its action in UTI:
110-kDa protein toxin induces actin reorganization into stress fibers and retraction fibers in human epithelial cells allowing them to spread, mediates cellular effects by the activation of small GTP-binding proteins. It stimulates PMNL cytotoxicity and enhances the effect on adherence to uroepithelial cells as well as the production of radical oxygen products. CNF-1 decreases the phagocytosis of PMNL cells and facilitates the growth of bacteria [(5)].

Aerobactin as an iron acquisition agent:
Iron acquisition is a critical requirement for UPEC survival in an environment that is iron-limited as the urinary tract. Aerobactin is one of the siderophore, a small-molecule iron chelators that are produced by UPEC strains to scavenge ferric iron (Fe^{3+}), an insoluble free cation, as free iron concentration are low in mammalian hosts. . Bacteria retrieve iron-bound siderophores through receptors that facilitate the transport of siderophore-iron complexes through the bacterial membrane and into the cytosol where the iron is released. Limiting iron availability is an important host defense against invading bacterial pathogens. These are some of the urovirulence characteristics that aid in colonization, host evasion and survival of UPEC strains [(6)].

Materials and Methods:-

Bacterial isolates:
The total of 212 *E. coli* isolated from urine samples were confirmed by biochemical tests based on standard bacterial identification.

DNA extraction:
DNA extraction was performed using boiling lysis method. Before DNA extraction, the *E. coli* isolates were cultured in LB broth at 37^{o}C for 18 hours. Bacteria were pelleted from 1.5 ml LB broth then suspended in 200 ml of sterile deionized water and incubated at 100^{o}C for 10 min. After centrifuging, the supernatant was used as template DNA and stored at -20^{o}C

PCR amplification:
PCR amplification of virulence genes were used to reveal the prevalence of three virulence genes hlyA, cnf, aer using specific primers. The amplification of virulence genes was carried out in a Thermal Cycler (Eppendorf Master) after standardizing the PCR conditions, consisted of initial denaturation at 94^{o}C for 2 min, followed by 30 cycles of denaturation at 94^{o}C for 1 min annealing at 60^{o}C for 30s and extension at 1 min 30s and final extension at 72^{o}C for 5min [(8)].

Table 1:-Virulence genes and its primer sequences

Virulence gene	Primer sequences (3' – 5')	Base pair size	Reference
Iron acquisition gene			
aer F aer R	AAACCTGGCTTACGCAACTGT ACCCGTCTGCAAATCATGGAT	556 bp	Codruta- Romanita et al.,2001
Toxin coding gene			
hlyA F hlyA R	AGATTCTTGGGCATGTATCCT TTGCTTTGCAGACTGTAGTGT	269 bp	Codruta- Romanita et al.,2001
cnf F cnf R	TTATATAGTCGTCAAGATGGA CACTAAGCTTTACAATATTGA	693 bp	Licznar et al., 2003

PCR product were then loaded in 1% agar gel electrophoresis and amplified DNA fragments were detected by UV fluorescence transilluminator and the size of the amplicons was estimated by comparing with 1kb DNA ladder included in the same gel.

Results:-

The results of molecular detection of specific toxin genes in *E. coli* were, aer (iron acquisition factor) accounting up to 107(50.5%), 74 (34.9%) and 33 (15.6%) isolates possess cnf and hlyA genes respectively. None of these were present in 73 (34.4%) of the isolates. The single isolate possess one gene or association of two or more genes, the expression of it optimally or sub-optimally makes the long persistence in the host and increase in severity of the infection.

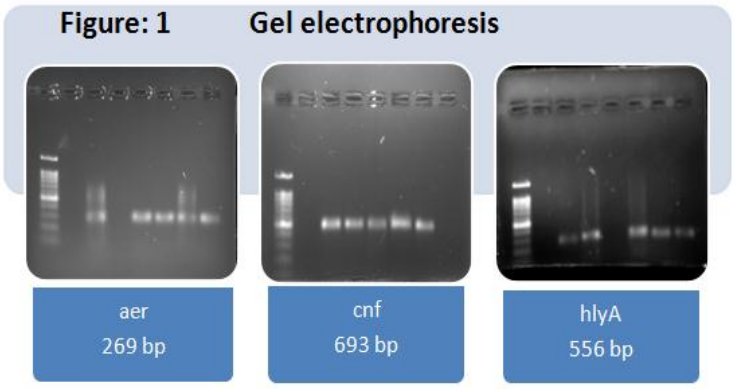

Figure1:-Agarose gel electrophoresis of aer, cnf, hlyA genes. L1 -100bp ladder, L2 – Negative control, L-3, 4,5,6,7,8 were the E. coli isolates

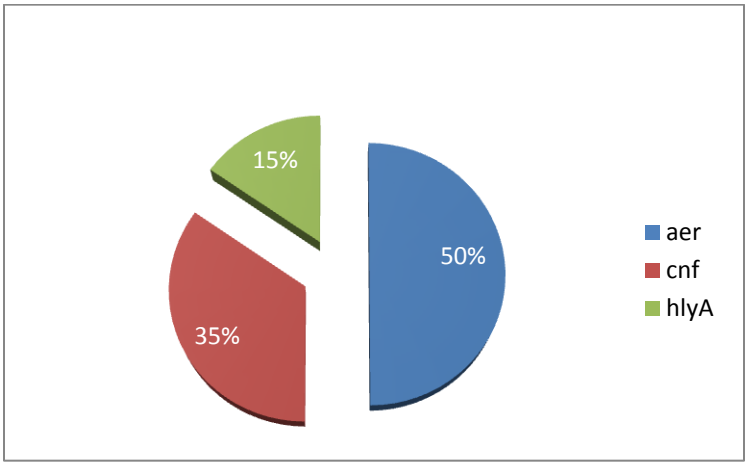

Figure 2:-Distribution of aer (aerobactin), cnf (cytotoxic necotising factor) and hlyA (hemolysin) genes

Virulence genes	**No. of isolates**	**%**
aer	47	22.2
cnf	22	10.4
hlyA	6	2.8
aer+cnf	37	17.5
aer+hlyA	12	5.7
cnf+hlyA	4	1.9
aer+cnf+hlyA	11	5.2
No genes	73	34.4

Table 2:-Distribution of individual and multiplegenes in a single *E. coli* isolate

Discussion:-

These aerobactin, hemolysin alpha and cytotoxic necrotizing factor act as both virulence and fitness factors. Virulence of the bacteria always depends on the survival and fitness of it inside the host environment [(14).] The quiescent state of the bacteria in the uroepithelial cells paves the long term persistence of it in the urinary tract. The exfoliation of infected urothelial cells plays dual role, firstly, it clears the infected cells and secondly, it disseminates the bacteria for establishment of infection in the rest of the urothelial cells.

HlyA (Alpha hemolysin), a pore forming toxin confers stable insertion into the epithelial and macrophage membranes which degrades the cyto-skeletal scaffolding paxillin protein and regulatory proteins of natural killer cell signaling pathway. HlyA induces the proteolysis of host proteins as well as modulates the function of epithelial cells, disables macrophages and finally inflammatory responses get suppressed (4).

CNF1 and HlyA are commonly found in UPEC isolates and are co-expressed as closely linked genes. CNF1 is a 115-kDa exotoxin has the role in pathogenesis of neonatal meningitis. Studies states that CNF has expressed in 31–44% of cystitis and 36–48% of pyelonephritis, it activates Rho GTPases, contributing to urothelial cell invasion by inducing the actin stress fiber formation, lamellopodia, filopodia and the inflammatory signal pathway gets modulated resutkting in cytotoxic effect (5).

aer (Aerobactin), a hydroxamate siderophore, produced maximally at low pH, has a higher Fe^{3+} binding stability in acidic environments(11). Iron is a known compound to increase the growth of the bacteria, highly expressed fitness factor commonly expressed in all UTI patients and negatively regulated by ferrous iron for the uptake of ferric in low iron conditions (12). It is an important factor in the progression of pyelonephritis (13).

Conclusion:-

These virulence factors play an essential role in uropathogenecity; also the frequency of its expression increases the severity of the disease with recurrent infection and may also be responsible for kidney damage and other extra intestinal manifestations.

References:-

1. Goñi FM, Ostolaza H. Braz J Med Biol Res. (1998): E. coli alpha-hemolysin: a membrane-active protein toxin.,31(8):1019-34.
2. Bhakdi S, Mackman N, Menestrina G, Gray L, Hugo F, Seeger W, Holland IB*(*1988*):* The hemolysin of **Escherichia coli**. *Eur J Epidemiol.,* 4*:*135–143.
3. Laura C.RistowRodney A.Welch. (2016): Hemolysin of uropathogenic *Escherichia coli*: A cloak or a dagger?Biochimica et BiophysicaActa (BBA) - Biomembranes., 1858(3): 538-545
4. Marianne Skals, Niklas R. Jorgensen, Jens Leipziger and Helle A. Praetorius. (2009):α-Hemolysin from *Escherichia coli* uses endogenous amplification through P2X receptor activation to induce hemolysis.,106 (10): 4030-4035.
5. Jason E. Michaud, KwangSikKim,WilliamHarty, Matthew Kasprenski,and Ming-Hsien Wang. (2017):Cytotoxic Necrotizing Factor-1 (CNF1) does not promote *E. coli* infection in a murine model of ascending pyelonephritis.,17: 127.
6. j r johnson, s l moseley, p l roberts, and w e stamm. (1998): aerobactin and other virulence factor genes among strains of escherichia coli causing urosepsis: association with patient characteristics. infect immun., 56(2): 405–412.
7. foroogh neamati ; farzaneh firoozeh ; mahmood saffari ; mohammad zibaei. (2015) virulence genes and antimicrobial resistance pattern in uropathogenic escherichia coli isolated from hospitalized patients in kashan, iran, j microbiol., 8(2): e17514.
8. maysaa el sayed zaki, ahmed elewa. evaluation of uropathogenic virulence genes in escherichia coli isolated from children with urinary tract infection. (2015): international journal of advanced research., 3(3):165-173
9. codruta-romanita u, damian m, tatu-chitoiu d, capusa c, fagaras r, tudorache d, et al. (2001): prevalence ofvirulence genes in escherichia coli strains isolated from romanian adult urinary tract infection cases. j cell mol med., 5: 303–10.
10. licznar p, eychenne i, aze´ma c, decramer s, bouissou f, fayet o, et al. (2003): revised prevalence of afa+ escherichia coli strains in acute pyelonephritis of children. pathol biol (paris)., 51:512–5.
11. Valdebenito M, Crumbliss AL, Winkelmann G, Hantke K. (2006). Environmental factors influence the production of enterobactin, salmochelin, aerobactin, and yersiniabactin in *Escherichia coli* strain Nissle 1917. Int. J.Med. Microbiol. 296:513–520.
12. M. Demir and I. KaleliPamukkale. (2004): Production by Escherichia coli isolates of siderophore and other virulence factors and their pathogenic role in a cutaneous infection model. Clinical Microbiology and Infectious Diseases, CMI., 10:1006–103.
13. Watts RE, Totsika M, Challinor VL, Mabbett AN, Ulett GC, De Voss JJ, Schembri MA. (2012): Contribution of siderophore systems to growth and urinary tract colonization of asymptomatic bacteriuria Escherichia coli.Infect Immun., 80(1):333-44.
14. SugandhaSrivastava, JyotsnaAgarwal, Bharti Mishra, RichaSrivastava. (2016): Virulence versus fitness determinants in Escherichia coli isolated from asymptomatic bacteriuria in healthy nonpregnant women. Indian Journal of Medical Microbiology., 34(1): 46-51