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Research Article

DETECTION OF NEUC GENE ENCODING CAPSULAR POLYSACCHARIDE AMONG THE URINARY ISOLATES OF ESCHERICHIACOLI FROM TERTIARY CARE HOSPITAL IN KANCHEEPURAM DIST

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ABSTRACT

Capsular polysaccharides (K antigens) are one of the main virulence marker for E. coli strains to cause urinary tract infections and neonatal meningitis. There are 50 chemically different capsular K antigens found, in which only a few are associated with infective E.coli strains.We have taken this study to detect the gene responsible for expression of K1 polysaccharide in E. coli isolates. 3/20 (15%) clinical isolate of urinary isolates of E.coli was found to harbour neuCgene.The pathogenicity of urinary tract infections by these pathogens might be crucially played by this gene encoding K capsular polysaccharide.

INTRODUCTION

Escherichia coli is a normal inhabitant of the gut micro flora. Some of the strains of E. coli cause severe human infections and many isolates of E. coli are found to produce extracellular polysaccharide capsules [1]. Capsular polysaccharides (K antigens) are one of the main virulence marker for E. coli strains to cause urinary tract infections and neonatal meningitis [2]. There are 50 chemically different capsular K antigens found, in which only a few are associated with infective E.coli strains. These polysaccharides associated with infections that are encoding with K1 antigen (polysialic acid) are involved in causing a wide variety ofinfections such as septicemia, urinary tract infections, and meningitis [3].The K1 antigen is one of the specific types of surface polysaccharidesfound among E. coli strains. There has been a vast difference in structural variability among E. coli capsules and at least 80 different K antigens were recognized so far. Only a few of these capsular structures have been implicated in pathogenesis of E. coli infections [1]. The pathogenicity of E. coli strains is directly correlated with the presence of numerous virulence factors [4]. The virulence of E. coli K1+ is related to the ability of the K1 capsule to inhibit phagocytosis and to resist antibody-independent serum bactericidal activity and the ability of K1+ strains to cross the blood–brain barrier (BBB) .Capsules protect bacteria against the host immune response. The α-2, 8-linked neuAc polysaccharides of E.coli Kl and Neisseriameningitidis group B resemble host glycoconjugates such as cell adhesion protein N-CAM[5]. The proteins necessary for synthesis, activation, and polymerization of neuAc are encoded by the 17-kbkps gene cluster of E.coli K1[6]. Silver et al have demonstrated that cells harboring mutations in neuC become sensitive to capsular polysaccharide specific phage only when supplied with exogenous sialicacid, suggesting involvement of the neuC gene product in neuAc biosynthesis[7].With this background, we have taken this study to detect the gene responsible for expression of K1 polysaccharide in E. coli isolates.

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MATERIALS AND METHODS

Bacterial isolates

A total of 20 non repetitive urinary isolates of Escherichia coli were collected from Saveetha Medical College and Hospitals, Chennai. They were processed for a battery of standard biochemical tests and confirmed. Isolates were preserved in semisolid trypticase soy broth stock and were stored at 4 ºC until further use.

Antibiotic susceptibility testing

Antibiotic susceptibility test was determined for these isolates to routinely used antibiotics such as ampicillin, amoxicillin, amikacin, norfloxacin, ceftazimide, cefotaxime, ciprofloxacin

and gentamicin, imipenem as by Kirby Bauer disc diffusion method [8].

Detection of neuCgene in E.coli

Escherichiacoli isolates were detected for the presence of neuCgene by PCR analysis. Detection of the gene was carried out using primer as depicted in table 1. Bacterial DNA was extracted by boiling lysis method. 1 µL of DNA extract was used as template for PCR reaction. The reaction mixture contained 1mM of $Mgcl_2$0.2mM dNTP mix and 0.8µM of neuC gene with 0.5U of Taq polymerase (New England Biolabs) in a 1x PCR buffered reaction. A positive control of E.coli with neuCgene was also included in this study. PCR amplification was carried out using thermal cycler (Eppendorf) with the following cycling condition. Initial denaturation at 96°C for 5 min and 30 cycles for 30s, 68°C for 30s and 68° C for 60s, followed by a final extension of 5 min at 74°C. PCR products were resolved in 1.5% agarose gel. A 100bp ladder was including in all the gel analysis [10].

Table 1 Gene sequencing of neuC gene

Primer	Primer sequence	Product size
neuC	AGGTGAAAAGCCTGGTAGTGTG GGTGGTACATCCCGGGATGTC	676 bp

RESULTS

Sample wise distribution of clinical isolates of E.coli

Of the 20 clinical isolates of E.coli, 12/20 (60%) were from acute urinary tract infections and 8/20 (40%) were from chronic urinary tract infections. Figure 1 depicts the sample wise distribution of clinical isolates of E.coli.

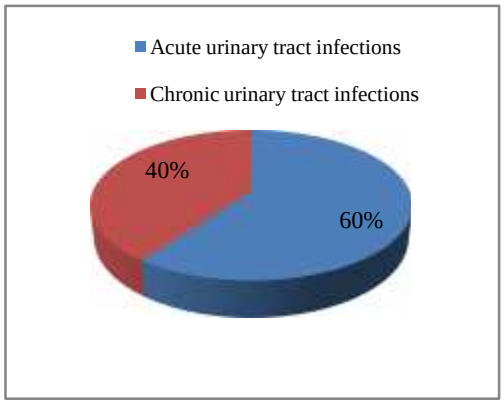

Figure 1 Sample wise distribution of urinary isolates of E.coli

Antibiotic susceptibility testing

In our isolates, we have found increased percentage 14/20 (70%) of isolates showed sensitivity to amikacin followed by gentamicin, which showed sensitivity of 9/20 (45%). 80- 90% of E.coli isolates showed resistance to cephalosporin group of drugs. 6/20 (30%) were found to be resistant to imipenem. However, we have observed an elevated level of resistance to other routinely used antibiotics. The detailed resistant pattern of E.coli isolates is shown in table 2.

Table 2 Showing antibiotic sensitivity pattern of E.coli

Antibiotics	Sensitivity(20) (%)	Intermediate (20) (%)	Resistant(20) (%)
Ampicillin	5	0	95
Amoxicillin	5	0	95
Ceftazidime	10	10	80
Cefotaxime	5	5	90
Amikacin	70	10	20
Gentamicin	45	20	35
Norfloxacin	15	15	70
Ciprofloxacin	20	5	75
Imipenem	70	0	30

Result ofneuCgene in E.coli

3/20 (15%) clinical isolate of urinary isolates of E.coli was found to harbour neuCgene.

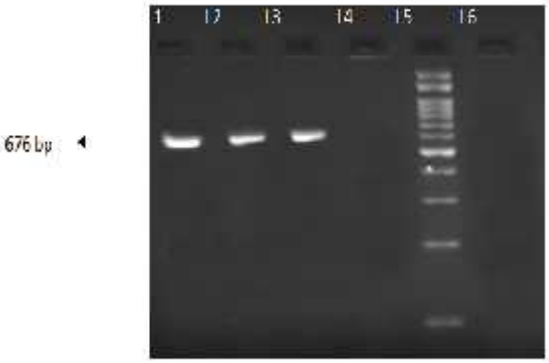

Figure 2 Representative gel picture showing positive for neuC gene

DISCUSSION

In our study we have seen 15% of our E. coli isolates from patients affected with urinary tract infections showed positive for neuC gene. This gene codes for capsular polysaccharide K antigen. This K antigen is found to regulate several functions in our system, among which resistance to phagocytosis is one of the main virulence function exhibited by this trait. Van Dijk et al showed that many of E. coli strains isolated from blood cultures positive patients with E. coli bacteremia possessed K antigen and were poorly phagocytized[9]. This inhibition of phagocytosis seems to be related to an impaired recognition of the K+ strains by the phagocytes due to ineffective opsonization then resistance to intracellular killing. In contrast, we have used only isolates from acute and chronic UTI. This might play a role in showing different results by this gene than isolates from blood samples. Similarly, study of study done by Kaczmarek et al in 2012 using capsular and non capsular strains of E. coli, they have reported 37.3% of positivity. But in our study we found great positiveness in showing this gene amplicons[10].

CONCLUSION

From our study, it is very clear that capsular polysaccharide is one of the main components in resisting the host immune response. The pathogenicity of urinary tract infections by these pathogens might be crucially played by this gene encoding K capsular polysaccharide. This is a basic and preliminary study. To rule out the relationship between presence of K capsular polysaccharide and urinary tract infections by E. coli, more number of samples and patients from different regions having UTI have to to be included.

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