DETECTION OF NEUC GENE ENCODING CAPSULAR POLYSACCHARIDE AMONG THE URINARY ISOLATES OF ESCHERICHIA COLI 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 neuC gene. 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. 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. These polysaccharides associated with infections that are encoding with K1 antigen (polysialic acid) are involved in causing a wide variety of infections such as septicemia, urinary tract infections, and meningitis. The K1 antigen is one of the specific types of surface polysaccharides found 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. The pathogenicity of E. coli strains is directly correlated with the presence of numerous virulence factors. 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. Capsules protect bacteria against the host immune response. The α-2, 8-linked neuAc polysaccharides of E.coli K1 and Neisseria meningitidis group B resemble host glycoconjugates such as cell adhesion protein N-CAM. The proteins necessary for synthesis, activation, and polymerization of neuAc are encoded by the 17-kb kbps gene cluster of E.coli K1. Silver et al. have demonstrated that cells harboring mutations in neuC become sensitive to capsular polysaccharide specific phage only when supplied with exogenous sialic acid, suggesting involvement of the neuC gene product in neuAc biosynthesis. With this background, we have taken this study to detect the gene responsible for expression of K1 polysaccharide in E. coli isolates.

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 °C until further use.

Antibiotic susceptibility testing

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

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

Detection of neuC gene in E.coli

Escherichiacoli isolates were detected for the presence of neuC gene 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 MgCl₂, 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 neuC gene 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   | AGTGGAAAGCCTGTAGGTTG | 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.

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               |
| Cefazidime  | 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 of neuC gene in E.coli

3/20 (15%) clinical isolate of urinary isolates of E.coli was found to harbour 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 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 be included.
Detection of neuc gene encoding capsular polysaccharide among the urinary isolates of escherichiacoli from tertiary care hospital in kancheepuram dist

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