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Detection of kps M II and tra T genes among E. coli isolates from asymptomatic bacteriuria.

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Abstract

Background: Escherichia coli, the commonest bacteria isolated in asymptomatic bacteriuria. Any bacteria to elicit symptomatic infection or asymptomatic colonization depend on the urovirulence genes and its expression. There are certain set of genes called as survival fitness genes that partly or solely involve in colonization of the bacteria in the urinary tract. Methods: The present study has detected the two survival fitness genes kpsMII and traT in E. coli isolated form asymptomatic bacteriuria using conventional PCR. **Results:** Among 30 E. coli isolated from asymptomatic bacteriuria 80% and 40% possess tra T and kps M II genes respectively. Conclusion: The knowledge on phenotype and genotype of these survival fitness genes should be analyzed in case of E. coli isolated from both symptomatic as asymptomatic bacteriuria to confine its role in pathogenesis.

Keywords: Asymptomatic Bacteriuria, E. Coli, Serum Resistance, Capsular Polysaccharide, Survival Fitness.

Introduction

Escherichia coli, a commensal of intestine also prevail as the most common microbe associated with asymptomatic bacteriuria (ASB). Among the different pathotypes of E. coli, Uropathogenic Escherichia coli causes' urinary tract infection with its Ur virulence factors like adhesins, toxins, flagella, host resistance etc., These virulence factors aids in functions like colonization, invasion and host defense mechanism inducing the infection[1]. Some commensal strains may diverge and colonize the urinary tract in the host with the absence of clinical manifestations. But the complete mechanism of asymptomatic colonization of this E. coli is yet under research.

The various virulence genes for adhesins like papC, papA, afa, sfa, the toxin genes cnf, hlyA have been studied in E. coli[2]. However, the knowledge on the

factors related to serum resistance as a defense mechanism is unclear. This serum resistance is a non-specific mechanism found in the serum of healthy humans against the bacteria, conferred by the cell surface components like capsule, proteins, polysaccharides etc.,[3,4] The individual or combined effect of capsular polysaccharide, surface proteins and O side chain antigens of certain LPS involve in defense mechanism. [5,6,7]

This serum resistance feature of E. coli from ASB makes it to persist in the host and gets decreased when there is loss of O antigens by the production of urine O antibodies by means of adaptive response in prolonged asymptomatic bladder colonization and progress to symptomatic UTI traT and kpsM II genes are the markers among urovirulence that affords phenotypic serum resistance expression.[8,9] In this study, E. coli from ASB were determined at molecular level for the presence of two genes traT and kpsMII encoding for serum resistance and capsular polysaccharide.

Materials And Methods

Study design: This study was a hospital based cross sectional study carried out in department of Microbiology during the period of October 2019 – January 2020 in a tertiary care hospital with the approval of Institutional Ethical Clearance. Urine samples were collected from 918 healthy subjects after getting written consent.

Inclusion and Exclusion criteria

Urine samples collected from the subjects without any symptoms of UTI are included and those subjects with UTI symptoms were excluded. [10].

Specimen collection, processing and bacterial identification

Midstream urine sample were collected after giving proper instruction to the subjects and processed according to standard culture methods. The bacteria isolated from asymptomatic patients were confirmed as ASB by collecting three consecutive urine samples, in which the same isolates with significant bacterial count was included in the study. Based on culture identification, biochemical tests were followed for the confirmation of E. coli isolates[10].

PCR amplification of survival fitness genes

The isolated microbial colonies were processed for molecular analysis. DNA extraction was done by boiling lysis method[11]. The conventional PCR was processed to detect the genes traT and kpsMII under temperature of 94°C for 1 s for denaturation, 63°C for 30 s annealing, 72°C for 90 s extension for 30 cycles and the final extension done at 72°C for 5mins for 1 cycle[12].

Table 1: Detection of protection genes[11, 12.]

Target gene	Primer sequences (5' to 3')	Size
Tra T	F: GGTGTGGTGCGATGAGCACAG	290 bp
	R: CACGGTTCAGCCATCCCTGAG	
Kps M II	F: GCG CAT TTG CTG ATA CTG TTG	272 bp
	R: CAT CAG ACG ATA AGC ATG AGC A	

PCR Cycling Conditions				
1 cycle	30 Cycles			1 cycle
Initial Denaturation	Denaturation	Annealing	Extension	Final Extension

94°C for 2mins	94°C for 1 s	63°C for 30 s	72°C for 90 s	72°C for 5mins
Hold at 4°C for 5 minutes				

The amplified PCR products were further preceded to agar gel electrophoresis in visualized under gel documentation system. The sizes of the PCR products were estimated by comparing their relative mobility with that of 100bp molecular marker.

Result

A total of 54 subjects were identified to be with asymptomatic bacteriuria out of 918 subjects. Among the different bacterial isolates E. coli 30 (55.6%) was found to be predominant.

The percentage of E. coli in less than 20 years was 28.1% in male and 25% in female followed by 12.5% each between the age group of 21- 40 years in female and 41-60 years in male as in figure 1.

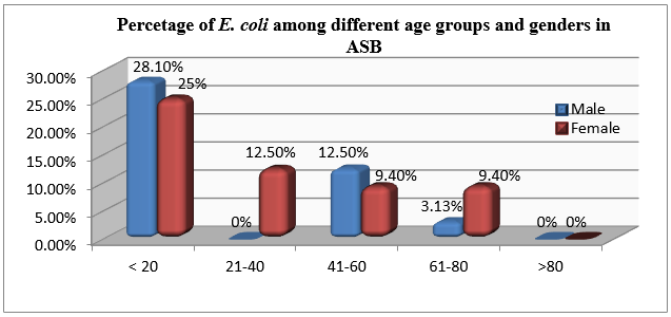

Figure 1: E. coli distribution in relation to different age groups among the genders in ASB.

In the molecular detection of traT and kpsMII genes, the present study identified 24(80%) and 12(40%) isolates with traT and kpsMII gene respectively out of 30 E. coli isolates. While comparing the distribution of the studied two genes among the age groups, traT and kpsMII genes were variably present in E. coli isolated from the different subjects of ASB in relation to the age groups.

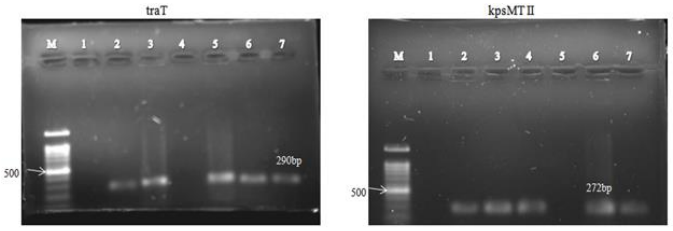

Figure 2: Molecular characterization of traT and kpsMII gene using PCR.

Discussion

These are the serum survival traT, a surface exclusion protein and kpsM II gene, a group II of capsular polysaccharide that confers resistance to bactericidal action by serum and protects the UPEC[12]. The outer bacterial surface coated by the capsule possesses antiphagocytic as well as anticomplementary effect making the immune recognition difficult.

Surface exclusion protein encoded by traT gene, an outer membrane lipoprotein with 25, 000 Dalton Mw and are associated non-covalently with muramin and exposed on the cell surface in fewE. coli[13]. This protein alters the complement component C3b binding site on the UPEC cell surface and interfere the assembly and attack of MAC complex.

Capsular polysaccharide is considered as the bonafide virulence of UPEC expressed at the outer cell surface encoded by kpsM II gene. Serum bactericidal action is proportional to the quantity of capsular polysaccharide[13]. There are very few studies on the genetic characterization of E. coli from ASB. The present study identified with 80% of traT and 40%of kpsMII among the total isolates. This is concordant to a study done by Srivastava et al[2]., with 79.1% traT except kpsMII genes with 64.1%. These genes were variedly present among the E. coli isolated from genders of different age groups, that showed its common survival ability. The expression of these protection/ serum resistance (survival) genes

have involvement in colonization of urinary tract but the signs and symptoms of UTI may get elicited depending upon the expression of other urovirulence genes.

Conclusion

These two survival fitness factor genes detected in E. coli from ASB in this study might involve in colonization of the bacteria based on their genetic expression. Since, it's has a survival function, may profoundly present in E coli isolated from symptomatic UTI, further research is required for its confirmation. The development of clinical manifestation involve other virulence genes too, yet the affirm activity of survival fitness factors in E. coli colonizing and causing symptomatic versus asymptomatic bacteriuria needs more genotypic and phenotypic characterization.

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