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Detection of siderophore production in uropathogenic *Escherichia coli* causing urinary tract infection in patients of Ujjain M.P. (India)

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ABSTRACT

Introduction: Urinary tract infection is most frequently found bacterial infection in urinary tract of human beings and 75-95% of urinary tract infections are caused by uropathogenic *E. coli*. The uropathogenic bacteria produces several virulence factors, siderophore is one of them. Siderophore is iron acquisition protein and play significant role in pathogenicity of UTI's by helping uropathogenic bacteria to persist in urinary tract of host cells in adverse conditions.

Materials and Methods: The study was conducted at SRL laboratory in Ujjain Center from November 2018- October 2019. Midstream urine sample of suspected UTI patients were collected and *E. coli* was isolated and identified using standard microbiological procedure and after incubation, growth was observed. Colonies more than 10^5cfu/ml were considered as significant for UTI and detection of siderophore production in *E. coli* uropathogenic bacteria was done by using Chrome Azurol Assay (CAS).

Results: Out of 200 collected urine sample in 120 urine samples uropathogenic *E. coli* bacteria was identified and isolated. Among 120 isolates of *E. coli* siderophore production was observed in 48 isolates of *E. coli*, and their percentage was 40%.

Conclusion: Iron acquisition protein or siderophores are a major virulence factor necessary for pathogenesis of UTI caused by *E. coli* and CAS assay is the most efficient method for detection of siderophore production in uropathogenic *E. coli.*

1. Introduction

Urinary tract is the second most common site for bacterial infection that occurs in any part of urinary tract. Urinary tract infection are frequently found infection in both gender and in all age groups of human being. Urinary tract infection is the most commonly found urological and renal disease caused by both gram negative and gram positive bacteria such as *E. coli,K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. saprophyticus* and *E. faecalis* but the disease is predominantly caused by *Escherichia coli* and it is responsible for up to 75% of all urinary tract infection and 95% of community acquired UTI.[1,2] *E. coli* colonize on periurethral area then enter into urinary tract and causes symptomatic disease called as uropathogenic *E. coli* (UPEC). The certain serotypes of *E. coli* associated with uropathogenicity.[3] The uropathogenic *E. coli* needed special features for active penetration, successful invasion and creating favorable environment inside the host cell. The bacteria obtained these features by expressing particular genes called virulence factors and these factors are responsible for converting commensal strain of *E. coli* into

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pathogenic strain. The important virulence factor of *E. coli* divided into two categories bacterial cell surface virulence factor and secreted virulence factor. Bacterial cell surface virulence factor includes fimbriae mainly type 1 fimbriae and P fimbriae, flagellum, capsular polysaccharide and outer membrane protein which are responsible for attachment and biofilm formation while secreted virulence factors includes haemolysin and siderophores. These secreted virulence factors help bacteria to colonize and persist into urinary tract and inactivate the functioning of defense system of host cells.[4] In pathogenic *E. coli* siderophore production is considered as major virulent factor. Siderophore is low molecular weight iron acquisition protein that helps bacteria in iron uptake, survival and colonization under adverse conditions. In all living organism including bacteria iron is needed for metabolism and growth. The bacterial cells require 10^{-7}-10^{-5} M iron for proper functioning, aerobic metabolism and multiplication. An *E. coli* bacterium uses iron for transporting and storing oxygen, DNA synthesis, electron transport and metabolism of peroxides. In host cell amount of free iron is limited and reduced during infection by pathogen. The host cell and infecting bacterial pathogens both compete for the limited bound iron in host tissue and organ systems.[5,6] In *E. coli* the hydroxymate siderophore (aerobactin) is the most effective iron chelation systems used by uropathogenic bacteria for iron acquisition to overcome the limited iron availability. In this system bacterial siderophore compete for iron with host cells iron binding protein, when iron bound with this siderophore, the iron is taken up by special receptors present on bacterial surface and then this iron utilized by uropathogens.[7,8] Several strains of *E. coli* responsible for causing urinary tract infection produces siderophores. The present study was designed to determine the production of siderophore urovirulence factor in *E. coli* uropathoenic bacteria isolated from patients of UTI in Ujjain.

2. Materials and Methods

The study was conducted in SRL laboratory Ujjain center for the period of one year from November 2018 to October 2019. During this period, midstream urine samples from suspected UTI patients were collected aseptically from different hospitals of Ujjain and processed in SRL laboratory. The collected 0.5 ml of urine sample was inoculated on three different selective and differential media, which were Blood agar, MacConkey agar and Chrome agar with the help of sterilized loop, by streak plate method. The plates were incubated at 37^oC for 24 h and after incubation colony forming unit were counted, if colony count is more than 10^5colony forming unit/ml than it indicated significant bacteriuria and was considered as positive urine culture. The single pure colonies were selected and subjected to morphological, microscopic and biochemical examinations as per the standard procedure for confirmation of isolated uropathogenic bacteria.[9] In morphological examination shape, size, color and margin of colony was observed. In microscopic examination gram staining was done for differentiation between gram positive and gram negative bacteria and the shape, color, arrangement of bacteria were also examined. The biochemical tests included Catalase, Oxidase, Coagulase, Indole production, Methyl Red, Voges-Proskauer, Citrate utilization, Triple sugar iron, Urease, Mannitol fermentation, Bile Esculine Hydrolysis test and motility tests. After confirmation of *E. coli* isolates detection of siderophore production was performed using Chrome Azurol Assay (CAS). In isolated *E. coli* detection of siderophore production was done according to method described by Vagarali in 2008.[10] In this method three types of solution were made, solution 1 prepared by dissolving 0.06 g CAS in 50 ml distilled water and then solution 2 was prepared for this 0.0027 gm of $FeCl_3$-6 H_2O added in 10 ml of 10mM HCl. The solution 3 prepared by dissolving 0.073 gm of HDTMA in 40 ml of distilled water after this solution 1 adds into 9 ml of solution 2 and then mixed with solution 3. The dark blue solution is produced which is autoclaved and poured into Petri plate. Modified CAS agar plate is prepared which was inoculated with isolated *E. coli* culture and incubated at 37^oC for 48 hours. After incubation, orange halo is formed around the each colony. The CAS assay detects color change of CAS-Iron complex from blue to orange after chelation of the bound iron by siderophores. The color changes from blue to orange due to production of siderophore.

3. Results

In total 200 urine samples of suspected patients in 120 urine samples *E. coli* bacteria was isolated. Among 120 isolates of *E. coli*, siderophore production was observed in 48 isolates and their percentage of occurrence was 40%. In siderophore producing *E. coli* orange hallo was detected around bacterial colony and color changes from blue to orange due to production of siderophore. The results are shown in Table 1 and Figure 1.

4. Discussion

Siderophores are one of the most important urovirulence factors of *E. coli* bacteria that helps bacteria in survival and multiplication inside host cells in iron starvation conditions by uptake of iron.[5,11,12] In the present study, In 120 confirmed *E. coli* isolates a very large number of *E. coli* isolates (40%) were found to be siderophore producers based on screening using CAS agar technique. Similar studies was carried out by other researchers and they also found large percentage of siderophore production in *E. coli* isolates.[13,14] In India Saleem and Daniel reported higher percentage of siderophore production in uropathogenic

Table 1: Siderophore production by uropathogenic *E. coli* isolates.

S.No .	Year of sampling	Total no. of collected urine sample	Number of *E . coli* isolates in urine sample (%)	Number of Siderophore producing E. *coli* isolates (%)
1	2018-19	200	120 (60.0)	48 (40.0)

Values in parenthesis indicates percentage.

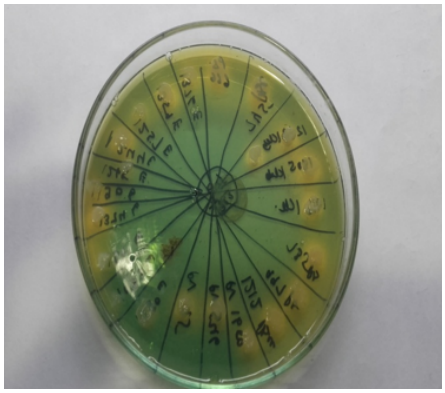

Fig. 1: Siderophore production in *E. coli* isolates.

E. coli in patients of type 2 diabetes mellitus.11 Some researchers observed higher siderophore production in patients with acute pyelonephrities. [15]

5. Conclusion

The present study indicated that siderophore is one of the most important virulence factor and play a major role in survival of uropathogenic *E. coli* bacteria in the iron-limited condition during the pathogenesis of UTI. The present study also revealed that screening of siderophore producers using CAS assay is important method employed for detection of pathogenic factors and useful in prevention and treatment of UTI.

6. Conflict of Interest

The authors declare no relevant conflicts of interest.

7. Source of Funding

None.

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