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Isolation and Identification of *Brevibacillus* and *Escherichia* spp. from Urinary Tract of Infected Patients

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ABSTRACT

Urinary tract infection (UTI) is a major public health concern, especially in women, across the globe. Among the uropathogens, *E. coli* is most implicated in UTI infected patients. Urine samples were collected, cultured and subcultured using different media to determine the morphology and biochemical properties of the bacteria in the samples. DNA samples from the pure cultures were amplified, sequenced and subjected to BLAST analysis. Isolates identified in the urine samples belonged to the genus *Escherichia*, *Klebsiella*, *Proteus*, *Enterobacter, Staphylococci* and *Brevibacilli*. The presence of *Escherichia marmotae* and *Brevibacillus panacihumi* was confirmed from the result of the BLAST. The study implicated *E. marmotae* and *B. panacihumi* in UTI infection in the studied population. Further study is encouraged to analyze the virulence genes responsible for their pathogenicity.

Key words : Urinary tract infection, uropathogens, molecular identification, brevibacillus

INTRODUCTION

Urinary tract infection (UTI) is the most prevalent and common infection affecting more than 15 million people worldwide. It is encountered by all the age groups from neonates to geriatric age group (Abad *et al.*, 2019). These infections are mostly observed in females when compared to males due to predisposition and proliferation of bacteria in urinary tract generally causing dysuria, fever, chills and frequent urination (Kalinderi *et al.,* 2018). Usually, UTI is the most common nosocomial infection caused by various groups of gram positive (+ve) and negative (-ve) bacteria such as species belonging to Enterobacteriaceae family, *Staphylococcus* sp., *Klebsiella* sp. and *Proteus* sp. Among these groups, 85-90% of the causal agent is represented by *E. coli* followed by other uropathogens.

On clinical grounds, density of 10^3 CFU/ml is referred for screening and diagnosis of UTI infection (Mehboob and Shuja, 2021). However, an increase in antibiotic resistance has enabled the use of broad spectrum of antibiotics. Antimicrobial resistance of uropathogens is the basis for UTI treatment, yet widespread use of antibiotics has resulted in bacterial resistance to specific medications. Several strains of *E. coli* which are referred as ESBL (Extended spectrum beta lactamase) were discovered to be resistant to tetracycline and ampicillin and hence, molecular based techniques provide precise information and identification of uropathogens from UTI infected patients (Tajbakhsh *et al.*, 2015; Farajzadah *et al.*, 2019). Many reports have implicated rarely classified Gram positive (+ve) bacteria as causal agent of complicated and uncomplicated urinary tract infection (UTI). This could be due to the lack of specific selective or non-selective culture media for isolation. Hence, much of emphasis is made on molecular based techniques to screen the rare unculturable bacteria. Therefore, the present study aimed at isolating uropathogens from urinary tract of infected patients and

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characterizing them on the basis of microscopic, chemical and molecular characterization.

MATERIALS AND METHODS

Total six urine samples were collected from G. B. Vaghani Pathological Laboratory, Surat between March to June 2020 and 1 ml of urine samples was inoculated into 9 ml peptone water for 24 h at 37^{0}C, then subcultured on nutrient agar, Mac Conkey agar, Blood agar, Hichrome Coliform agar, and Eosin Methylene Blue agar (EMB) and incubated at 37°C for 24 h in upright position. Pure cultures were obtained and identified using both morphological characteristic and biochemical tests.

The selected pure cultures isolated from urine samples were subjected to isolation of DNA by phenol chloroform method. The bacterial cultures were inoculated into Luria broth and were kept at 37°C for 24 h. The cultures were then used for extraction of DNA and were maintained at 20°C for 16S rDNA PCR. PCR amplification was carried out with the set of universal primers using BDT Cycle Sequencing Kit on ABI 3500xl Genetic Analyzer. BLAST was performed to know the phylogenetic relevance from an existing microbial DNA database using NCBI (Hamzah and Khan, 2017).

RESULTS AND DISCUSSION

A total of eight morphologically distinct isolates (Fig. 1) were screened and identified from the urine samples collected from human urinary tract infected patients. The isolates were identified phenotypically based on microscopic and biochemical analysis. The principal biochemical tests such as indole, methyl red, Voges-Proskauer test, citrate utilization test, triple sugar iron and sugar fermentation tests were performed to identify the bacterial isolates. It was observed that the isolates belonged to genera : *Escherichia, Klebsiella, Proteus, Enterobacter, Staphylococci* and *Brevibacill* as per the standard protocol. Their biochemical characteristics are presented in Table 1. Similar studies have also reported prevalence of *E. coli*, *Proteus* and *Klebsiella* as UTI pathogens (Flores-Mireless *et al.*, 2015; Gradwohl *et al.*, 2016). Further,

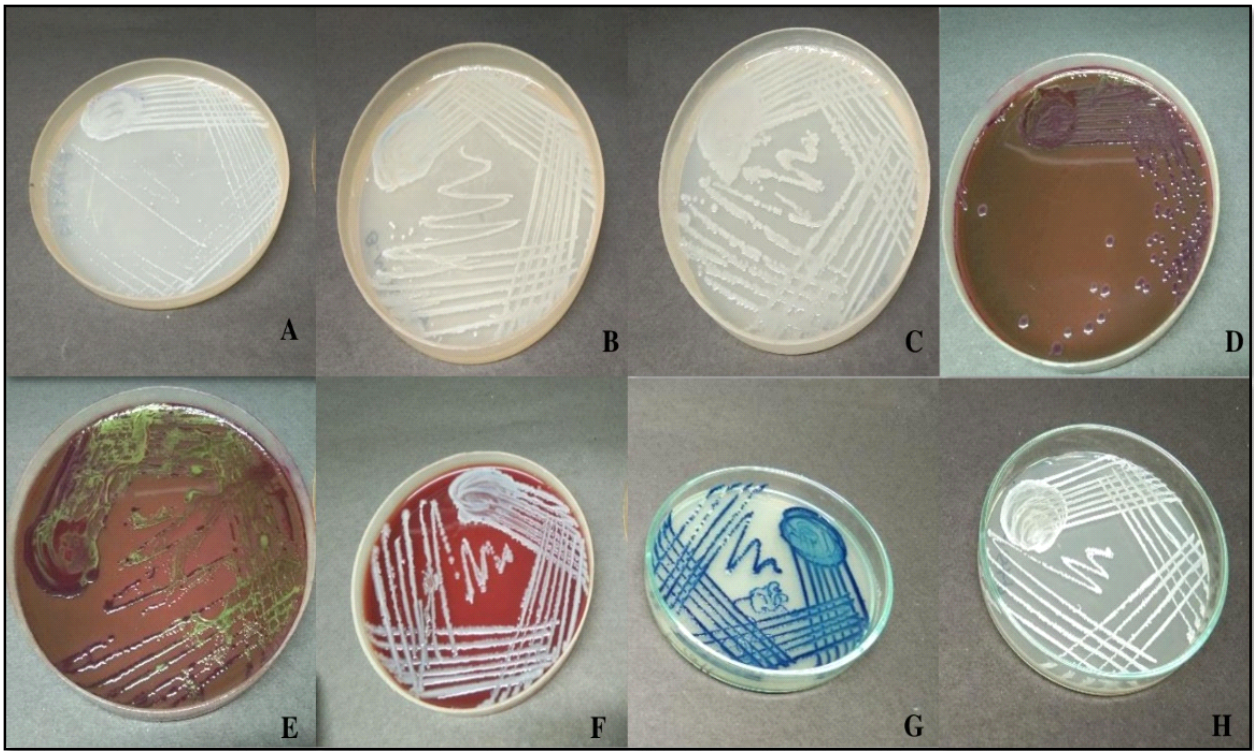

Fig. 1. Isolates from urine samples on specific media : (A) *Staphylococcus* on nutrient agar, (B) *Klebsiella* on nutrient agar, (C) *Proteus* on nutrient agar, (D) *Enterobacter* on EMB agar, (E) *E. coli* on EMB agar, (F) *Proteus* on blood agar, (G) *E. marmotae* on hichrome coliform agar and (H) *Brevibacilli* on nutrient agar.

Table 1. Biochemical characteristics of isolated obtained from urine samples

Test		*E. coli*	*Klebsiella*	*Proteus*	*Enterobacter*	*Brevibacilli*	*Staphylococci*
Indole		+	-	+	-	-	-
Methyl-red		+	-	+	-	+	+
Voges proskauer		-	+	-	+	-	+
Citrate utilization		-	+	+	-	-	+
Triple sugar iron test		+	+	+	+	+	+
Sugar fermentation	Lactose	+	+	-	-	-	-
	Glucose	+	+	+	+	+	+
	Mannitol	+	+	-	-	-	+
	Fructose	-	+	-	+	+	+
	Xylose	-	+	-	-	-	-

+ : Positive and - : Negative.

the isolates that were repeatedly obtained on isolation were identified to be *Escherichia marmotae* and *Brevibacillus panacihumi* on the basis of phylogenetic analysis with 99% similarity (Figs. 2 and 3). Mostly, *Brevibacillus* species were found to be isolated from soil samples but incidence of *Brevibacillusagri* from UTI infected patients was reported by Suneeva *et al.* (2014). Generally, the most common UTI pathogens infecting 80 to 90% of females were found in the range of *Escherichia*> *Enterococcus*> *Staphylococcus*> *Klebsiella*>*Proteus* (Hamzah and Khan, 2017; Vranic *et al.,* 2017; Garuba *et al.,* 2021). For the first time, it was observed that *Brevibacillus panacihumi* was also responsible for causing UTI and it can be further identified to screen virulence factors responsible for pathogenicity.

CONCLUSION

The present study was carried out to isolate and identify the uropathogens responsible for UTI which provided a means of accounting the infective role of *Escherichia marmotae* and *Brevibacillus panacihumi* with that of other pathogenic bacteria. Furthermore, virulence genes responsible for pathogenicity could be traced to get better understanding of these newly identified strains and henceforth, it will help in treatment of UTI infection.

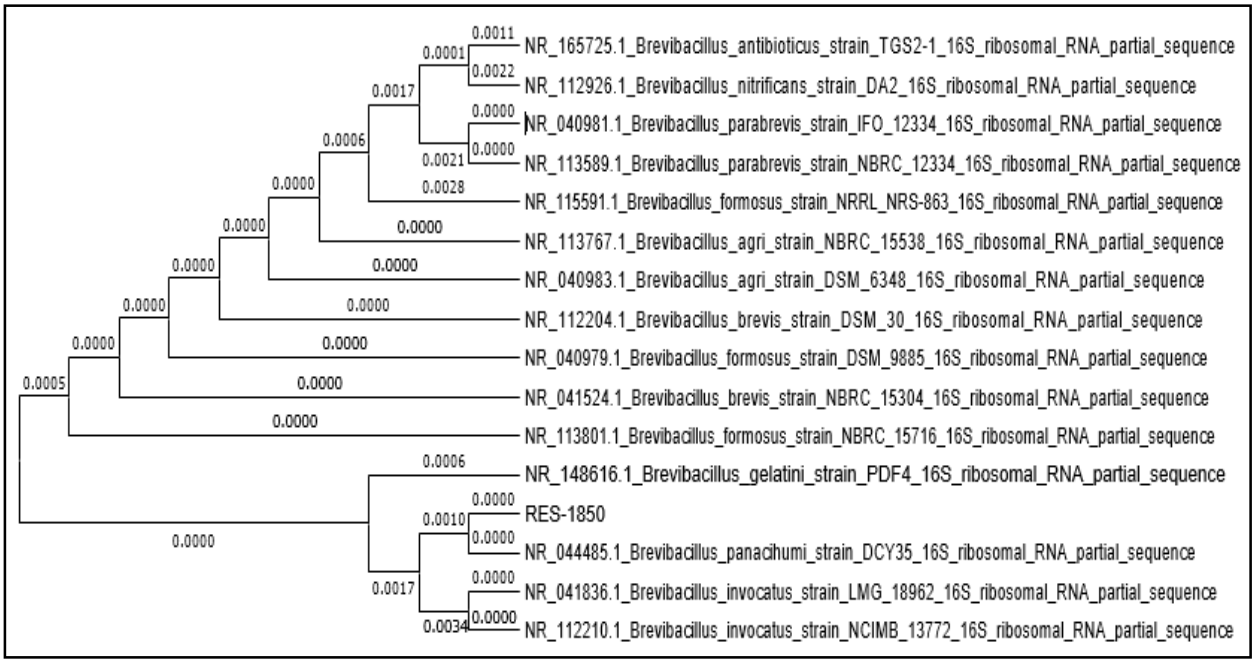

Fig. 2. Phylogenetic tree, based on neighbour-joining, derived from an alignment comprising C 5′ end partial region sequences suggesting isolate as *Brevibacillus panacihumi* (Sequence ID : NR_044485.1).

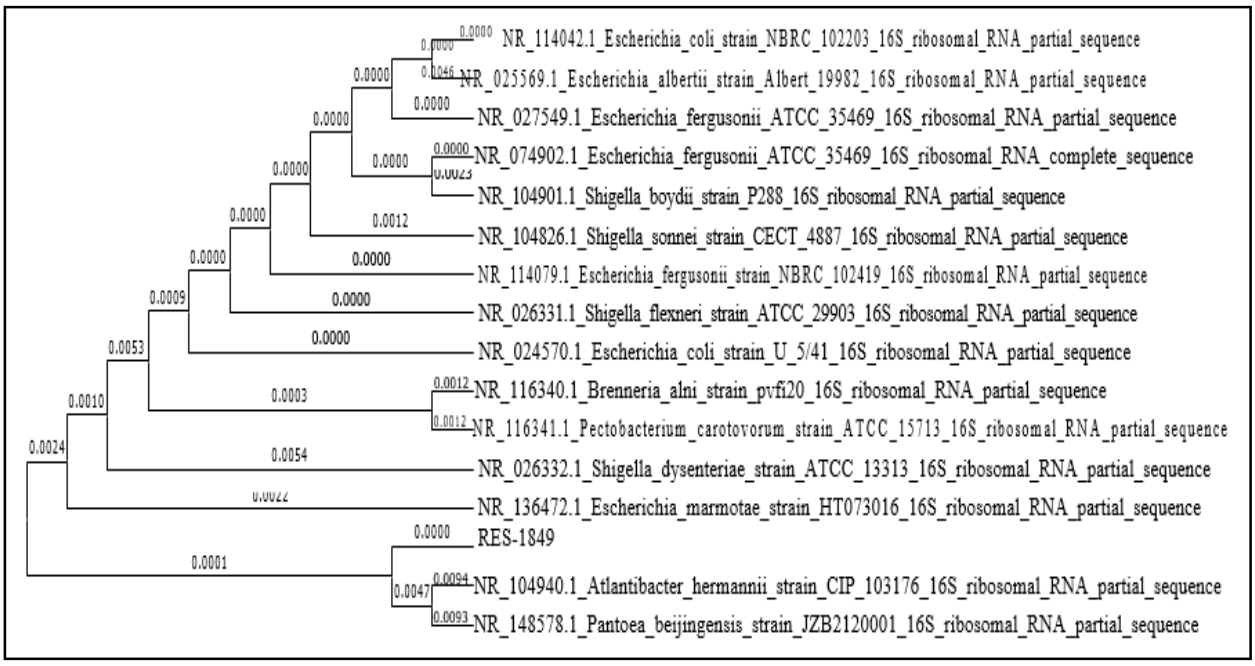

Fig. 3. Phylogenetic tree, based on neighbour-joining, derived from an alignment comprising C 5′ end partial region sequences suggesting isolate as *Escherichia marmotae* (Sequence ID : NR_136472.1).

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