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Isolation of Uropathogens from Pediatric Associated UTI, with Special Focus on the Detection of *Proteus Vulgaris*

Basima Q. Hassan AL-Saadi [1] , Sanaa J. Kadhum[1], Seham Hamel Muhaiesen[1]

[1] Institute of Genetic Engineering and Biotechnology for Postgraduate, University of Baghdad

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Abstract: The study was conducted in AL-Kadhumia hospital for children, AL-Kadhumia city / Baghdad, through period April-July 2014. Mid –stream urine of 75 patients were cultured. Out of these 67(89.33%) patients` urine were positive for bacteriological culture. The distribution of infection was 50.7% infants aged 2-9 months and 46.3% children aged 1-4 years. Total of six bacterial species isolated were *E.coli* 35(52.2%), *Enterobacter spp*. 16(23.9%), *Proteus spp*.11 (14.9%), *Klebsialle spp*. 3(4.7%), *Pseudomonas spp*. 1 (1.5 %) and *Staphylococcus spp*. 1(1.5 %). The isolated organisms were identified based on conventional bacteriological and biochemical analyses and were characterized. PCR technique was used to detect *Proteus vulgaris* by using species – specific primers Urease C, the results of primer urease C observe 3 isolate were positive. Rapid diagnosis of the pathogen in a clinical sample is always very important.

Key words: uropathogens, pediatric, UTI, *proteus vulgaris*, urease C.

Corresponding author: should be addressed (Email: basima22000@yahoo.co.uk)

Introduction

The urinary tract infection is one of the common sites of bacterial infection in human. Urinary tract infection (UTI) is among the most frequently occurring human bacterial infection accounting for about 20% of all infections acquired outside hospitals (1). Almost 9% of the UTI are ascending with bacterial gaining access to the urinary tract via the urethra to the bladder and then to the upper part of the urinary tract (2). Infants and children are also susceptible to UTI, febrile UTI in children tend to be associated with vesicaureteral reflex and the potential for renal scarring, and pediatric UTI might predispose patients to adult disease. It has been reported that in USA about 50% of all urinary tract pathogens belong to the enteric group, according to data from US centers' for disease control and prevention about 30% of all identified nosocomial pathogens belong to this groups (3). More than 90% of UTI are caused by a single bacterial species, *E.coli* is the most frequent infecting organism in acute infection, *Klebsiealla*, *Staphylococci*, *Enterobacter*, *Proteus*, *Pseudomonas*, and *Enterococci* species are more often isolated from patients (4,5). *Proteus* species is known uropathogen especially causing urinary tract infection in catheterized patients and in those with urinary tract abnormalities, it may lead to pyelonephritis, stones, fever and bacteremia (6). Individualization of pathogenic strain is essential to study of

association between clinical case and possible source of infection and for this purpose various typing methods have been devised. Convential methods that include antibiotic resistance patterns, biochemical reactions, bacteriocin typing and phage typing are usually in efficient, time consuming and expensive (4). More recently, there has been increasing interest in the application of molecular techniques to type bacterial pathogen; polymerase chain reaction has been the most successful techniques that are rapid and sensitive (4, 7).
The aim of present study is to focus on isolate, identify and characterize pathogenic agents involving UTI, and application of PCR techniques in diagnosis of *Proteus vulgaris* from samples containing various types of bacteria by using specific primers.

Materials and Methods

1-Urine Samples

Mid-stream urine (MSU) specimens were collected under a septic condition in asterial tubes (5-15 ml). Collection of MSU samples from young children is difficult and can be realized with a help of catheterization of the urethra. MSU specimens immediately transported to the laboratory. In a total of 75 patients admitted to AL-Kadhumia Hospital for young children in center of Kadhumia city /Baghdad, with signs and symptoms suggestive of UTI during the period from April- July 2014, the age of the patients ranged from two months to four years. Urine specimens were cultured for isolation of microbial agents within one hour of sampling on blood agar, MacConkey agar and on brain heart infusion broth (BHIB). A single colony were isolated from primary positive cultures and identified according to the criteria of (8,9,10). The pure culture was maintained in BHI agar slant with glycerol 15 % for further analysis (9).

2-Identification of Clinical Isolates

Cultural characteristics: Grams` stain was performed for the clinical isolates for studying the microscopic properties as initial identification of bacterial isolates (10). All gram negative isolates were identified using commercial systems, Bio- Merieux Api -20E (9). Isolates giving discrepant results were submitted to the identification according to Bergeys` Manual of systemic bacteriology (8). All organisms identified presumptively as *proteus spp*. were tested for indole production , urease test , Methyl red (MR), Voges-Proskauer (VP) test ,Citrate utilization test, Gelatin hydrolysis test, H_2S production test, Carbohydrate fermentation test and Urease test by using Api-20 E (Bio-Merieux, France) (9).

3- DNA Extraction from *proteus spp.*

Genomic DNA was extracted from *proteus* spp. isolates by a commercial nucleic acid extraction kits (Geneaid, Korea) according to manufacturers` instructions. The isolated DNA was checked by 1 % agarose electrophoresis and viewed using UV illuminator.

4- PCR for Confirmation of *proteus vulgaris* Bacteria

For conformation by PCR different sets of primers targeting specific genes were

used (11, 12). The PCR conditions were as recommended by the respective uthers (Bioneer, Korea) using PCR reaction mixtures prepared in a 0.2ml Eppendorf tube with 20 full reaction volumes, which contained the following : 1 µl of upstream primer, 1µl of downstream primer , 2µl DNA template, and 16µl of nuclease free water. The primers used in this study were synthesized by (Bioneer, Korea) are described in (table 1).

Table (1) primers set used for detection of *proteus* spp. (Bioneer, Korea)

Primers	Sequences(5`-3`)	Size (bp)	Bacteria	Reference
16s rRNA	F GGA AAC GGTGGC TAA TAC CGC ATA AT R GCA GCG CTA GGT GAG CCT AAT GGG	101	Proteus spp.	11
Urease C	F CGCTTT GCG ATG GCA AGT ACA AGT AAG R GCA AAT TGA GTG ACT TTG GCT GGA CC	263	Proteus vulgaris	12

The reaction mixture was prepared by mixing the components in sterile Eppendr of tubes (0.5 ml), which were then centrifuged at 30 minutes, and then the Eppendorf tubes were closed and transferred into a thermo cycler (Applied Biosystem ,USA) for product detection, 7 µl of the PCR mixture was subjected to electrophoresis in a 2% agarose gel.

The PCR Cycle for 16S rRNA Used As Follows

40 cycles of denaturation at 95°c for 30Sec., annealing at 60°c for 30Sec., extension at 72°c for 10Sec., final extension at 72°c for 10minutes.(11)

PCR for Urease C Gene of *P. vulgaris* Used As Follows

40 cycles were carried out: denaturation at 95 C for 1 min, annealing at 62°c for 1.3 min. and extension at 74°c for 1 min., after last cycle tubes were incubated at 74C for 7min. (12)

Results and Discussion

The result showed that, 67 (89.33%) patients out of 75 were to be urine culture positive. There were 34 (50.7%) infants aged 2-9 months and 31(46.3%) children aged 1-4years with urine sample positive culture. Gram negative bacilli accounted for 66(92.2%)of positive culture, while Gram positive cocci were 1.5% , the frequency of isolated uropathogens and their relation to age is given in table (2).

The age distribution of patients in our study is agreed with those other reported studies, showing predominance of infants (70.7%) with UTI (13).The

high prevalence of infection among infants is related to many environmental conditions and host factors such as health care, socioeconomic standards and hygiene practices in each community. The prevalence of gram positive cocci was not high in our study in contrast the Enterobacteriaceae family were the most microorganism isolated from UTI accounting 95.2% of total isolated bacteria and amongst them *E. coli* was the most predominant bacteria (52.2%), this study is agreed with those other reported studies showing that *E.coli*, is the most common cause of UTIs with frequency rate of 59% (14).

Table (2) the frequency of bacterial agents isolated from urine specimens and their relation to age

Isolated bacteria	Number (%)	Infant (%) (2-9months)	Child (%) 1-4 years
E.coli	35(52.2%)	20(57.1%)	15 (42.7%)
Enterobacter spp.	16(23.9%)	6(37.5%)	10(62.1%)
Proteus spp.	11(14.9%)	7(63.6%)	4(36.3%)
Klebsialle spp.	3(4.7%)	2(66.6%)	1(33.3%)
Pseudomonas spp.	1(1.5%)	1(100%)	O (0%)
Staphylococcus spp.	1(1.5%)	0(0%)	1(100%)

Proteus Isolation and Identification

The result indicated that 11(14.9%) isolates of *Proteus spp*. collected from urine sample, only three of them were confirmed as *proteus vulgaris*, one from infant and two from young children as associated nosocomial infection (15).

Motility was exhibited by the three isolates when grown on agar plate and non – lactose fermented (NLF) on MacConkey agar, urease and Indol production, it produce positive result for sulfur reduction, tryptophan deaminase production, gelatinase activity, sacchorose fermentation and negative results for remainder tests, table (3).

Table (3) Biochemical tests for *Proteus vulgaris*

Biochemical tests	*Proteus vulgaris*
motility	+ (swarming)
MacConkey	NLF
Indol	+
Urease	+
MR	+
VP	-
H2S	+
Gelatinase activity	+
Tryptophan deaminase production	+
Sacchorose fermentation	+

+ means presence, - means absence, MR= Methyl red, VP= voges –proskauer

Api 20E was used to confirm the results of identification and the result of Api 20E come with those obtained in traditional tests (8, 9). The result obtained in this study were not in agreement with local study which indicated that the rate of isolates were 7%, 8%, 18 % respectively, (13,16,17). The uropathogens identified in our study are similar to those of many other studies conducted in different countries either in region or internationally, however different results have been reported. (5, 14, 18).

PCR for the Confirmation of *proteus* spp.

A polymerase chain reaction plays an important role as a powerful tool in clinical microbiology studies and has been widely applied to detect bacteria and genus of interest. In this study genomic DNA was extracted from growth culture (figure 1).

Attempts were made to extract DNA from urine sample directly, but they did not give reliability in amount of DNA, for this reason, relied on the growth culture. The results are shown in (figure 2). All 11 isolates of *proteus* spp.were confirmed through PCR by targeting 16S rRNA (11, 15, 19, and 20). In our study 16S rRNA based primer used to reach accurate diagnosis and differentiate closely related species 16S rRNA gene is the center of bacterial classification (19). However, it is known that 16S rRNA gene is highly conserved which possess the question if this gene is suitable to differentiate very closely related species. Furthermore several studies have described the results obtained by PCR with 16S rRNA (20, 21).For the present study this gene was chosen because it appears to be conserved in all *proteus spp*. (13, 22) but not for *proteus vulgaris* as this gene (101bp) is high specific for *proteus mirabilis* (11, 12 ,13) as shown in (figure 2.)

In this study Urease C based primers used to reach rapid and accurate diagnosis of *proteus vulgaris* bacteria and the result were excellent with first primer Urease C 263bp which positively resulted for all urine sample , were DNA originated from bacteria which grown in BHIB as shown in (Figure 3) , However these results demonstrated that using the designed primers, the 263 bp DNA fragment only appeared in PCR product using *proteus vulgaris* cell as a template and thus the Urease C based PCR method can be used for specific detection of *proteus vulgaris (*12) .

There are no previous studies done in Iraq for molecular detection of *proteus vulgaris*. However, molecular techniques has over Convential methods is that it can provide result in 24hs` whereas routine culture followed by biochemical tests need 36-48hs` (7)

All samples in routine examination in this figure get positive result for growth of *Proteus vulgaris*

Figure (1): Genomic DNA of *Proteus spp*. On 1 % agarose gel electrophoresis (70 voltage \hours) (Lanes 1-8) DNA extracted from isolated bacteria

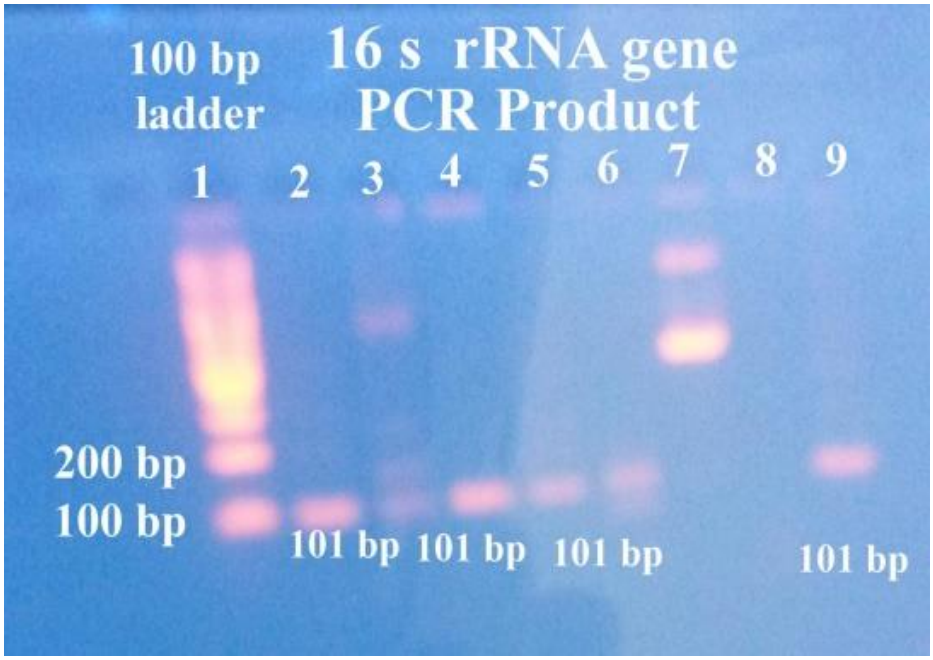

Figure (2): Agarose gel electrophoresis of PCR of 16S r RNA amplicon
Lane 1 ladder (100bp), Lane 8 negative control
Lane, 2, 3, 4, 5, 6, 7,9 isolates of *Proteus spp.*

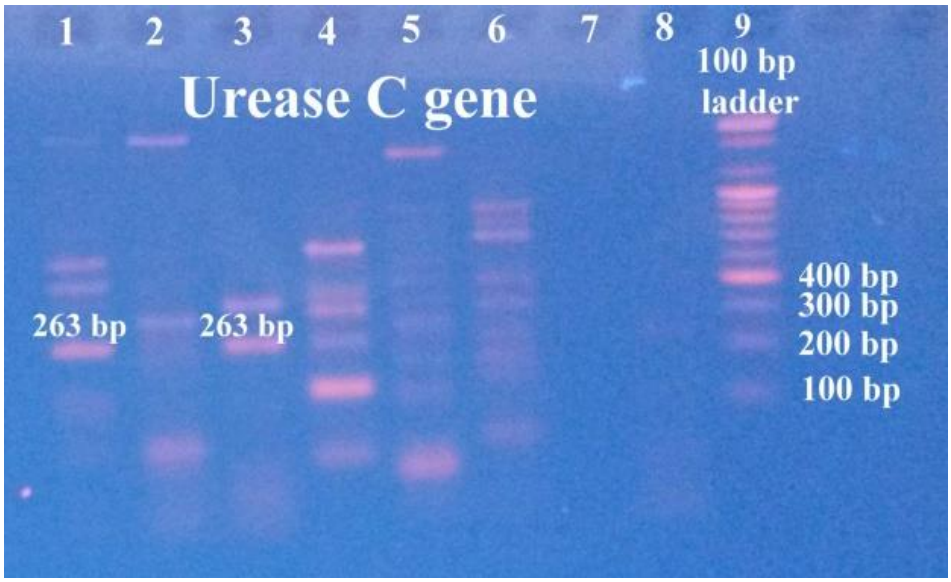

Figure (3): PCR product of primer Urease C (bp263) with DNA extracted from the samples enriched in BHIB.
Lane 9= Ladder 100bp
Lane 8= Negative control
Lane 1, 3, 4 = our isolate represent *Proteus vulgaris* 263bp

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