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ORIGINAL ARTICLE

Evaluation of the efficiency of URO-QUICK™ System in the Detection of Urinary Tract Infections and use of Fluorescence In-Situ Hybridization (FISH) In Detection of *Escherichia coli* in Urine

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ABSTRACT

Key words:
URO-QUICK™ System, Fluorescence In-Situ Hybridization (FISH), Escherichia coli and Urine

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Background: *Urinary tract infection (UTI) is outlined as a significant number of patho organisms within the urinary system. The URO-QUICK system relies on a light-scat technique that steadfastly detects microbial growth in fluid samples, giving real-time g curves and bacterial counts (cfu/ml). The system was initially designed for the fast screen urine samples. The sensitivity of the system, expressed in terms of cfu/ml, depends on the ti detection, therefore if it is intended to detect 100, 00cfu/ml, and the time required for dete is 180 min. Early detection of microbial pathogens is crucial for rational and conser antibiotic use particularly within the case of known local resistance patterns. Fluorescer situ hybridization (FISH) with rRNA-targeted oligonucleotide probes enables the fas specific detection of individual microbial cells from urine samples.* ***Objective:*** *to evalua URO-QUICK system in comparison with conventional culture methods and utilize fluores in situ hybridization (FISH) for early detection of E .coli in urine* ***Methodology:*** *A total of urine samples were collected from different hospitalized patients in Urology & Nephr Center, Mansoura University. The specimens were cultured by standard methods. 0.5 well-mixed urine was inoculated in Uro-Quick broth vials which were subsequently appl the Uro-Quick instrument and incubated for a maximum of 3h.* ***Results****: 214(21%) samples positive by conventional culture; bacterial identification was performed with automated V 2 system. 113(52%) samples were E. coli, 36(16.8%) samples were Klebsiella pneum 19(8.9%) samples were Pseudomonas aeruginosa, 5(2.4%) samples were Proteus mir 3(1.4%) Enterobacter cloacae, 2(0.9) samples were Morganella morganii, 1(0.5%) sampl Citrobacter freundii, 1(0.5%) sample was Acinetobacter baumannii, 19(8.9%) samples Enterococcus faecalis, 2(0.9%) samples were Streptococcus pneumoniae, 2(0.9%) sa were Staphylococcus aureus, and 11(5.1%) samples were Candida albicans. 220(2 samples were positive by Uro-Quick Screening test. Miacom's uriFISH Screen for E. coli for the complementary positions targeting position 342–357 in E. coli 16S rRNA CTGCTGCCTCCCGTAG-3'). From 220 positive samples by Uro-Quick Screening Miacom's uriFISH Screen for E. coli yielded only 113 positive samples from urine sa* ***Conclusion:*** *The Uro-Quick screening system seems to be a reliable instrument to obtain microbiological results in a timely manner and FISH technique able to specifically pathogens quantitatively in situ even in samples containing mixtures of bacteria.*

INTRODUCTION

Urinary tract infection (UTI) is one among the foremost common bacterial infections of humans and a significant cause of morbidity. UTI additionally accounts for 25 to 40% of all nosocomial infections, so these infections are considered crucial medical and financial load on health care systems [1]. The limit for significant bacteriuria depends upon presence/absence of symptoms, bacteria category, number of species isolated, method of specimen collection and gender [2].

Most urinary tract infections (UTIs) are caused by facultative anaerobes in the bowel flora. The majority of pathogens are gram-negative bacteria, with the prevalence of members of family Enterobacteriaceae [3]. Apart from *E. coli* which is by far the commonest organism, other gram-positive organisms such as *Enterococcus faecalis* and *Staphylococcus saprophyticus* are responsible for most community-acquired infections [4].

UTI typically starts as a bladder infection whoever can, depending on the bacterial strain, ascends to the kidneys and may ultimately lead to renal failure or dissemination to the blood. A variety of microorganisms entering the urinary tract establish bacteriuria often at levels more than or equal to 10^5 colony forming units of

bacteria/ml of urine. Numerous studies reported that hospital-acquired UTI is approximately 33% [3].

E. coli was the predominant pathogen triggering bacterial urinary tract infection, followed by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis* [5].

The natural habitat of *Escherichia coli* is the intestinal tract of humans and animals. It is thus considered as an indicator for fecal contamination of water and foods. *E. coli* is that the most frequent contributing infectious agent in human microbial infections. Extra intestinal infections include urinary tract infections, which occur when the tract is obstructed or spontaneously caused by the pathovar uropathogenic *E. coli* (UPEC). The fore most vital different coli infections are cholecystitis, appendicitis, peritonitis, postoperative wound infections, and sepsis. *E. coli* bacterial infections are detected by means of pathogen identification [6].

A technique for the fast and specific identification of individual microbial cells inside their natural environments has been long anticipated. Most microorganisms have terribly restricted morphological detail, preventing the visual identification likely with plants and higher animals [7].

The URO-QUICK™ system (now HB&L) is based on a light-scattering technique that dependably detects microbial growth in fluid samples, providing real-time growth curves and bacterial counts (cfu/ml). The system was mainly designed for the fast screening of urine samples. However, the basis of the detection (nephelometric detection of light scattering) potentially makes the system more widely applicable. The sensitivity of the system, expressed in terms of cfu/ml, depends on the time of detection, so if it is intended to detect 100,000cfu/ml, the time required for detection is 180 min. Similarly, the incubation must be prolonged to 235 min or more (up to 6 h) if the detection cutoff is lower than 1000 cfu/ml (6 h <50 cfu/ml) [8]. The system was evaluated here in comparison with conventional culture methods. Fluorescence in situ hybridization (FISH) was developed to quantitatively detect a specific group of microorganisms, including those yet to be cultivated, at the single cell level under a microscope [9,10].

Fluorescence in situ hybridization (FISH) permits the identification of target microorganisms (bacteria, yeasts and protozoa) at genus or species level because of the binding of short, about 18 to 25 basepair-sized, fluorescence-labeled target-specific oligonucleotide probes to the ribosomal RNA with subsequent investigation under the fluorescence microscope. FISH probes for numerous indications are deposited within the database “probeBase” [11,12]. Once fixation on typical slides and sample preparation (e.g. by preceding target-specific permeabilization steps), hybridization is performed, whereby the precise probe binds to complementary ribosomal RNA of the target organism. Excess probes are removed in a washing step [13].

FISH is presently one amongst of the key techniques utilized in microbial ecology and environmental microbiology in addition to in public health [14-16].

FISH-based studies on microorganisms in humans and plants have recently attracted a lot of consideration [17,18]. The aim of this work is to evaluate the URO-QUICK system in comparison with conventional culture methods and utilize fluorescence in situ hybridization (FISH) for early detection of *E .coli* in urine.

METHODLOGY

Samples Collections:

A total of 1016 urine samples were collected from different hospitalized patients in Urology and Nephrology Center, Mansoura University. These samples were collected in sterile tightly sealed containers under aseptic precautions during collection process. The study protocol was approved by the ethics committee, Faculty of Medicine, Mansoura University.

Microbiological culture and identification of isolates:

A quantitative culture was performed to assess the significance of the potential pathogens recovered. A bacterial growth of$\geq 10^5$ colony forming units (cfu/ml) was considered indicative for a clinically relevant pathogen. All samples were vortexed, and 1 µl of the specimen was inoculated in agar media using a calibrate loop. Culture media included CLED agar, sheep blood agar for bacteria and Saboureaud dextrose agar for fungi. Samples were incubated at 37 °C for 48 h in aerobic conditions. Microbial culture on agar plates (assumed to be the gold standard for the isolation of the pathogens) was used to evaluate the HB&L™system performance. Isolates were identified by VITEK® 2 System (BioMérieux, Marcy-I' Etoile, France) with the Gram Negative card (**GN card**) for Gram-negative and the Gram Positive card (**GP card**) for Gram-positive bacteria.

Uro-Quick system:

0.5 mL of well-mixed urine was inoculated in Uro-Quick broth media vials which were then applied to the Uro-Quick instrument and incubated for a maximum of 3 h (standard protocol) according to the manufacturer's instructions. Every 5 min the optical density, which indicates bacterial growth, was measured by the system. Based on optical density measurement the colony forming units were calculated. A colony forming unit of $\geq 1 \times 10^5$/mL was considered positive for bacteriuria.

Fluorescence in situ hybridization (FISH):

Miacom's uriFISH Screen for *E. coli* was performed according to manufacturer's instructions; the following probe was used for the complementary positions targeting position 342–357 in *E. coli* 16S rRNA (5’-CTGCTGCCTCCCGTAG-3’). 2 ml from urine sample was centrifuged; the sediments were suspended in 500

µl of clinical sample buffer, 10 µl of the dilution was added on each of two wells of the microscope slide, pre-warmed to 52°C for 5 min. 10 µl of reconstituted Lysis Solution was added, pre-warmed to 52°C for 5 min. The slides were transferred to ethanol bath for 5 minutes, place on the hotplate and allow ethanol to evaporate. 10 µl of reconstituted Beacon Solution was added, incubated for 10 minutes at 52°C and the slides were transferred to the stop bath for I min. One small drop of Mounting Medium (approximately 5µl) was added. The probe-conferred signals were observed using **a Nikon E800 epifluorescence microscopy**.

RESULTS

Culture of urine specimens was used as the gold traditional technique. Among 1016 urine samples, only 214 samples were positive by conventional culture, 220 samples were positive by Uro-Quick instrument (Table 1). During the test periods, the culture revealed 21% UTIs, and 79.4% culture-negative urine samples.

From 220 positive samples by Uro-Quick Screening test, Miacom's uriFISH Screen for *E. coli* yielded only 113 positive samples from urine samples.

In comparison to the results obtained by culture techniques, the Uro-Quick showed a Sensitivity 100 %, Specificity 97.3 % NPV (Negative predictive value) 100 % PPV (Positive predictive value) 97.27 % for HB&L.

Table 1: Comparison between conventional culture methods and HB&L

Conventional culture	HB & L			
	Growth	No Growth	Total	%
	No	No	No	
Growth	214	----	214	21
No Growth	----	796	796	78.4
Contamination	6	-----	6	0.6
Total	220	796	1016	100

The bacterial identification performed with automated VITEK 2 system showed that 113 samples were *E. coli* (52%), 36 samples were *Klebsiella pneumoniae* (16.8%), 19 samples were *Pseudomonas aeruginosa* (8.9%), 5 samples were *Proteus mirabilis* (2.7%), 3 *Enterobacter cloacae* (1.4%), 2 samples were *Morganella morganii* (0.9%), one sample was *Citrobacter freundii* (0.5 %), 1 sample was *Acinetobacter baumannii* (0.5%), 19 samples were *Enterococcus faecalis* (8.9%), 2 samples were *Streptococcus pneumoniae* (0.9%), 2 samples were *Staphylococcus aureus* (0.9%), 11 samples were *Candida albicans* (5.1 %) (Table 2).

Table 2: Bacteria and yeast identification results by VITEK 2.

Gram	Organisms	No	%
Gram negative bacteria	*Escherichia coli*	113	52
	Klebsiella pneumoniae	36	16.8
	Pseudomonas aeruginosa	19	8.9
	Proteus mirabilis	5	2.4
	Enterobacter cloacae	3	1.4
	Morganella morganii	2	0.9
	Citrobacter freundii	1	0.5
	Acinetobacter baumannii	1	0.5
Gram positive bacteria	*Enterococcus faecalis*	19	8.9
	Streptococcus pneumoniae	2	0.9
	Staphylococcus aureus	2	0.9
Fungi	*Candida albicans*	11	5.1

The biochemical identification tests in Gram-Negative (GN) cards in the VITEK 2 system are designed as every three tests are read and sixteen digit biotype numbers are generated by assigning a weighted numerical value to all positive reactions for each isolate. Those positive tests in every three tests in horizontal rows are summed to get digits, the first positive test gets a value of 1, positive tests in the second get a value of 2, and positive tests in the third get a value of 4. All negative reactions are scored zero. The biotype number is determined by adding the values for every three horizontal tests. The three tests positive are digit by 7 by summation the first test positive digits by 1, the second test positive digits by 2 and the third test positive digits by 4. When this procedure has been reported for all horizontal rows, the biotype is completed (tables 3 and 4).

Table 3: Biotype (1) 0405610550026601; the number of this biotype is 50 isolates.

Biochemical Details																	
2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
33	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	-	37	MNT	-	39	5KG	-
40	ILATK	-	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	IHISa	-	56	CMT	+	57	BGUR	+
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	+	64	ILATa	-			

Table 4: The 113 (52%) isolates of *Escherichia coli* were divided into five biotypes.

No.	Biotype	No. of *E.coli* isolates	%
1	0405610550026601	50	44
2	0405610550524610	20	18
3	0405611550526611	18	16
4	0405611540507611	15	13
5	0407610554526611	10	9
Total		113	100

Fig. 1: *E. coli* on CLED agar after 24 hr at 37°C

Fig. 2: *E. coli* on Blood agar after 24 hr at 37°C

Figures 1, 2 showed *E. coli* on **CLED** (Cysteine Lactose Electrolyte-Deficient) agar. It is a type of differential medium recommended for diagnostic urinary bacteriology this medium supports the growth of all urinary potential pathogens and provides distinct colony morphology and Columbia blood agar. On CLED *E. coli* show yellow, opaque colonies with a slightly deeper colored center (Non-lactose fermenting strains – blue colonies) yellow medium. On blood agar *E .coli* show white mucoid colonies.

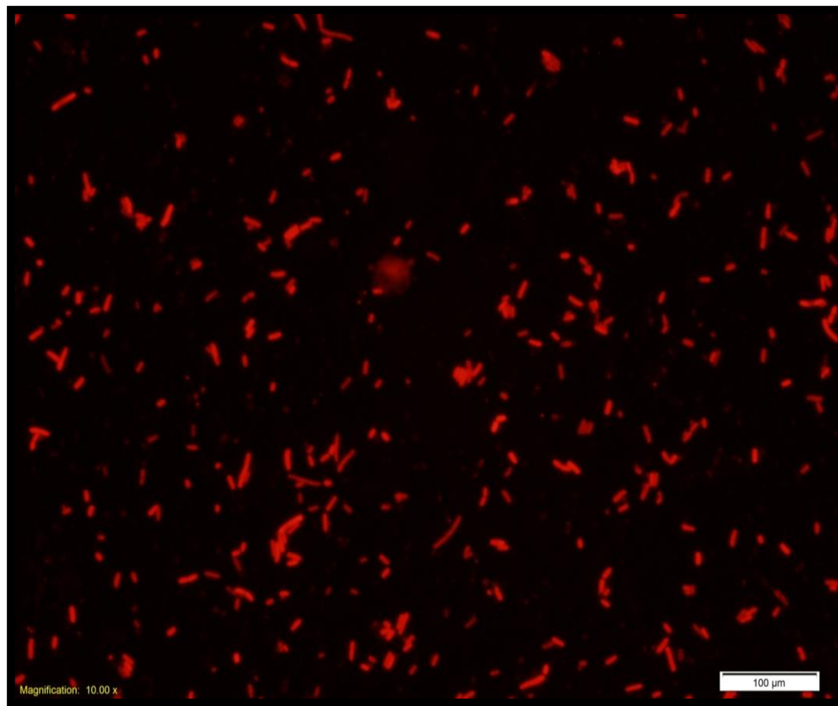

Fig 3: Positive of *E. coli* by fluorescent microscopy

FISH Miacom's uriFISH Screen for *E. coli* yielded 113 positive samples

The result in figure 3 showed the bacterial cells of *E. coli* which appeared as fluorescent cells under the fluorescent microscope due to fluorescence labeled molecular probe (beacon). It consist of a DNA folded into a hair pin-like structure linked to a fluorophore to one end and to a quencher on the other end. This structure when enter the bacterial cell wall and membrane binds to r RNA targets of *E. coli*, then the fluorophore of the beacon is spatially separated from its quencher and is able to emit light if it is excited with adequate light source. By this fluorescent signals one can observe the cell's intact morphology.

DISCUSSION

It is important to provide a rapid diagnosis of UTIs and to reduce costs. Standard culture methods require incubation of at least 24 up to 48 h. so we aimed to establish a system that enables us to rapidly identify the sterile urine samples and exclude them from long and expensive culture procedures [19].

Urine samples comprise the largest volume of workload in routine bacteriology laboratories, but almost 80% of these turn out to be culture negative. Despite the availability of many commercial screening systems, there is no agreement on which system seems to be ideal for routine microbiology laboratories. Ideally such a system ought to in agreement with conventional cultures to a great extent with regard to accuracy. On the other hand, such a system should be easy to perform, reproducible and have a considerably shorter turnaround time [20].

The advantage of fast automated systems is assumed to be the potentiality of rapid identification of sterile urines and thus the exclusion of UTIs. The hands-on time using the automated systems was considerably reduced to about 30 s per sample, as compared to that of the culture method. However, the most saving in time is obtained for negative samples. In order to gain information about antibiotic susceptibility, the positive tested samples have to be analyzed by culture techniques anyway [19].

The main goal of diagnostic microbiology is the rapid and correct identification of microorganisms in their natural habitats. Culture-based methods are typically too selective, particularly for fastidious or yet-to-be cultured bacteria, and therefore these methods do not reflect the precise composition of mixed bacterial communities or microbial diversity in infections more over they are time consuming. [21,22].

Only few studies estimated the sensitivity and specificity of the Uro Quick system. These studies stated a total sensitivity ranged between 83% to 93%. [23,24]; but, they did not investigate the sensitivity and specificity of the different UTI-causing microorganisms and in some studies, fungi were even excluded [24]. this the present, we recorded a total sensitivity of 100% and Specificity 97.3 %, which might be based on cut-off value high colony forming units (10^5/mL) of microorganisms, of the Uro-Quick system.

Our results showed that, *E. coli* (52%) was the most predominant followed by *Klebsiella pneumoniae* (16.8%) this agrees with other studies performed as found in the study of Keegan, S.J.; 2003, [25], on 93strains, where *E. coli* was the most isolated microorganisms (59%) and *Klebsiella pneumoniae* was (14%).

Fluorescent in situ hybridization (FISH) is a well-established non–culture-based procedure for identifying bacteria that uses fluorescence labeled- oligonucleotide probes based on 16S rRNA sequences specific to the species, genus, or other phylogenetic levels of interest[26].

Therefore we aimed at establishing a system that enables us to rapidly identify the sterile urine samples and exclude them from time-consuming and expensive culture procedures.

In situ hybridization allowed the detection of the bacterial cells of *E. coli* urine samples without false positive reaction. FISH can be used to identify bacteria on different phylogenetic levels. Specific oligonucleotide probes and primers have been designed for many bacterial species. In comparison to cultivation-based methods for describing community structure; hybridization with molecular probes is rapid and more reliable. Due to its low costs and ease of use, FISH will remain a useful technique for some time in developing countries.

CONCLUSION

The Uro-Quick screening system seems to be a reliable instrument to obtain urine microbiological results in a rapidly. Considering the rapidity with which the URO-QUICK™ system achieved the identification of positive specimens in this study (3 h), the approach holds great promise for early detecting and identifying microbial pathogens.

Our results encourage consideration of the routine use of the URO-QUICK™ system in the culturing of biological samples as an efficacious alternative to the conventional approach.

No other method is as rapid, reliable, simple and cheap as FISH and able to specifically detect pathogens quantitatively in situ even in samples containing mixtures of bacteria.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.

- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. Ross V, Klemm P. Global gene expression profiling of the asymptomatic bacteriuria *E. coli* strain 83972 in the human urinary tract. Infect Immun. 2006; 74: 3565-3575.
2. Nicolle L E. A symptomatic bacteriuria when to screen and when to treat. Infect. Dis. Clin. North Am., 2003; 17: 367-394.
3. Rahman F, Sadia C, Rahman M, Ahmed D, Hossain A. Antimicrobial Resistance Pattern of Gram-negative Bacteria Causing Urinary Tract Infection. S. J. Pharm. Soci., 2009; 2(1): 44-50.
4. Elsotohi E. Clinical Significance of Bacteriuria in Patients with Orthotopic Sigmoid Neobladder after Radical Cystectomy. Afr. J. Urol.2009; 15(4):1110-5704.
5. Manikandan S, Ganesapandian S, Singh M, Kumaraguru AK. Antimicrobial Susceptibility Pattern of Urinary Tract Infection Causing Human Pathogenic Bacteria. AJMS. 2011; 3 (2) :56-60.
6. Kayser FH, Biensz KA, Eckert J, Zinkernagl RM. Medical Microbiology Handbook. Georg Thieme Verlagu, Ru¨ digerstraßle. 2005; 14, 70469 Stuttgart, Germany (2005).
7. Bohlool BB, Schmidt EL. The immunofluorescence approach in microbial ecology. Adv Microb Ecol, 1980;4:203-241.
8. Fujitani S, Yu LV. Quantitative cultures for diagnosing ventilator-associated pneumonia: a critique. Clin Infect Dis.2006; 43: 106–13.
9. Amann RI, Binder BJ , Olson RJ, Chisholm SW, Devereux R, Stahl DA. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microbiol.1990; 56:1919–1925.
10. Amann R, Fuchs BM. Single-cell identification in microbial communities by improved fluorescence *in situ* hybridization techniques. Nat. Rev. Microbiol. 2008; 6:339–348.
11. Loy A, Horn M, Wagner M. probeBase: an online resource for rRNA-targeted oligonucleotide probes. Nucleic Acids Res. 2003; 31:514–16.
12. Loy A, Maixner F, Wagner M. probeBase–an online resource for rRNA-targeted oligonucleotide probes: new features 2007. Nucleic Acids Res.2007; 35:D800–4.
13. Moter A, Gobel UB. Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. J Microbiol Methods.2000; 41:85–112.
14. Lawrence J, Korber D, Neu T. Analytical Imaging and Microscopy Techniques, p. 40–68. *In* C. Hurst, R. Crawford, J.Garland, D. Lipson, A. Mills, and L. Stetzenbach (ed.), Manual of Environmental Microbiology, 3rd ed. ASM Press, Washington DC.2007
15. Nielsen PH, Daims H, Lemmer H. FISH Handbook for Biological Wastewater Treatment: Identification and Quantification of Microorganisms in Activated Sludge and Biofilms by FISH. IWA Publishing, New York.2009
16. Wendeberg A. Fluorescence *in situ* hybridization for the identification of environmental microbes. Cold Spring Harb. Protoc.2010 1:pdb.prot5366.
17. Attar N. FISHing in the oral microbiota. Nat. Rev. Microbiol. 2016; 14:132–133.
18. Berg G, Grube M, Schloter M, Smalla K. Unraveling the plant microbiome: looking back and future perspective. Front. Microbiol.2014 5:148.
19. Matthias Marschal, Matthias Wienke, Steffen Hoering, Ingo B. Autenrieth, Julia-Stefanie Frick. Evaluation of 3 different rapid automated systems for diagnosis of urinary tract infections. Diagnostic Microbiology and Infectious Disease. 2012; 72 (2012) 125–130.
20. Arzu Ilki, Pınar Bekdemir, Nurver Ulger, Guner Soyletir. Rapid reporting of urine culture results: impact of the uro-quick screening system. NEW MICROBIOLOGICA,2010; 33, 147-153.
21. Wagner M, Amann R, Lemmer H, Schleifer, KH, Probing activated sludge with proteobacteria-specific oligo-nucleotides: inadequacy of culture-dependent methods for describing microbial community structure. Appl. Environ. Microbiol. 1993; 59, 1520–1525.
22. Choi BK, Paster BJ, Dewhirst FE, Gobel UB. Diversity of cultivable and uncultivable oral spirochetes from a patient with severe destructive periodontitis. Infect. Immun.1994; 62, 1889–1895.
23. Cermak P, Ptakova M, Kubele J, Cermakova R, Jedlickova A. Detection of urinary tract infection using Uro-Quick system and quantitative dilution method. Klin Mikrobiol Infekc Lek.2009; 15:166–170.
24. Ilki A, Bekdemir P, Ulger N, Soyletir G. Rapid reporting of urine culture results: impact of the Uro-Quick screening system. N Microbiol.2010; 33:147–153.
25. Keegan SJ, Graham C, Neal DE, Blum-Oeher G, N'Dow J, Pearson, JP, Gally DL. Characterization

of *E. coli* Strains Causing Urinary Tract Infection in Patients with Transposed Intestinal Segments. J. Urol. 2003; 169 (6): 2382-2387.

26. Bottari B, Ercolini D, Gatti M. Application of FISH technology for microbiological analysis: current state and prospects. Appl Microbiol Biotechnol. 2006;73:485–494.