BIODIVERSITAS
Volume 19, Number 4, July 2018
Pages: 1375-1381

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d190425

Diversity of urinary tract infection bacteria in children in Indonesia based on metagenomic approach

GRACE CHRISTINE, SRI BUDIARTI♥, RIKA INDRI ASTUTI
Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor. Jl. Agatis, Dramaga Campus, Bogor 16680, West Java, Indonesia. Tel./fax.: +62-251-8622833, ♥email: s_budiarti@yahoo.com

Manuscript received: 7 March 2018. Revision accepted: 26 June 2018.

Abstract. *Christine G, Budiarti S, Astuti RI. 2018. Diversity of urinary tract infection bacteria in children in Indonesia based on metagenomic approach. Biodiversitas 19: 1375-1381.* Urinary tract infection (UTI) is a common bacterial infection in childhood in both males and females. The infection is usually caused by bacterial invasion of the urinary tract including the lower and the upper urinary tract. In Indonesia, to the best our knowledge, the diversity of urinary tract infection bacteria has not been reported yet. Therefore, the aims of this study were to identify the diversity of both culturable and unculturable bacteria in children diagnosed with UTI. In this study, urine samples were obtained from different age groups ranging 6-17 years. Analysis of 16S rRNA gene sequence showed eight culturable isolates (SBU1, SBU2, SBU3, SBU4, SBU5, SBU6, SBU7, SBU8) are closely related to *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Citrobacter* sp. and *Acinetobacter* sp. with maximum identity up to 98-99%. Diversity of unculturable bacteria community based on 16S rRNA gene was represented by 9 DGGE (Denaturing Gradient Gel Electrophoresis) bands. The nine respective bands showed the similarity ranging from 84 up to 96% with *Klebsiella* sp, *Escherichia* sp., *Lactococcus lactic*, *Shigella flexneri* and uncultured bacterium. Based on phylogenetic analysis, all culturable isolates belong to phylum Proteobacteria, which is dominated by family of Enterobacteriaceae. Interestingly, by using metagenomic approach, it is observed that bacteria belong to phylum Firmicutes were found in the UTI-diagnosed patients, in addition to those bacterial isolates from phylum Proteobacteria. To our knowledge, this is the first study to report the occurrence of Firmicutes and Proteobacteria in UTI-diagnosed patients in Indonesia.

Keywords: Bacteria, DGGE, UTI, urine, 16S rRNA

INTRODUCTION

Urinary Tract Infection (UTI) is an infection of the urinary tract caused by invasion of pathogenic microorganisms (Mireles et al. 2015). This diseases is one of the important causes of morbidity and mortality in children, and crowned as the second infectious disease which often attacks children after upper respiratory tract infection (Najar et al. 2009). The prevalence of UTI occurrence has different presentations for both men and women. Based on research conducted by Hidayah et al. (2011), in Indonesia, from 200 children aged 1-10 years who were evaluated suffering from UTI there were about 33% men and 67% women. The main cause of UTI is mainly due to contamination of the urinary tract by normal perineal flora (rectum). In addition, factors that trigger infection also caused by abnormalities in the urinary tract (Minardi et al. 2011). According to World Health Organization (WHO 2011), bacteria as the main cause of UTI in children are derived from Enterobacteriaceae group. This group of bacteria is generally a normal microbiota in human, especially in the gastrointestinal tract, but under certain circumstances, these bacteria can be pathogenic and cause infectious diseases including UTI (Giske et al. 2011). Some species of this group have the ability to enter the bladder, and form biofilms that are resistant to the immune response (Salvatore et al. 2011).

The early prevention approach to UTI disease is by maintaining sanitation or personal hygiene and antibiotic therapy. However, the use of irrational antibiotics would contribute to antibiotics resistance leading to the more severe infection-mediated bacteria. Another solution to prevent UTI is by vaccinations. Vaccination is an act of vaccine (antigen) that stimulates the formation of immunity (antibodies) of the immune system. However, to date, the development of vaccines to prevent UTI is limited. One of the obstacles is the microbial community profile that may involve in UTI infections is not revealed comprehensively. Moreover, the fact that 99% of these bacteria cannot be cultured under laboratory conditions (Kimura 2006), while only 1% of them can be cultured, making it more difficult to develop the target of action of that UTI-vaccines. In Indonesia, the diversity of UTI bacteria has not been reported yet, thus studies diversity of both culturable and uncultured bacteria in UTI patients need to be conducted. This study aimed to analyze the diversity and abundance of culturable and unculturable bacteria in the urinary tract of the UTI patients. To have better understanding of the microbial community of both culturable and unculturable bacteria, we applied two different approaches, i.e., cultivated-based techniques for culturable bacteria, and metagenomic approach through Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) based on the 16S gene rRNA for unculturable bacteria. This study may use to develop strategy for vaccine development in preventing UTI disease in Indonesia.

MATERIALS AND METHODS

Sample collection

Thirteen urine samples of children (10 samples of UTI-diagnosed and 3 non-UTI as control samples) from different age groups ranging 6-17 years (Table 1), were collected from pediatric ward of Indonesia Red Cross Hospital, Bogor, West Java. Urine samples were transported to the Laboratory of Microbiology, Department of Biology, Bogor Agricultural University. Urine samples were kept in sterile container and held at 4-8ºC then processed within 24 hours after collection.

Isolation and characterization of cultured bacteria

Cultured bacteria were isolated by serial dilution and spread plate techniques. About 1 mL urine sample was serially diluted to final dilution of 10^{-3} by using 0.85% NaCl solution. About 0.1 mL suspension from each dilution was spread on to Nutrient Agar (NA) and Eosin Methylene Blue Agar (EMBA) medium and incubated at 37°C for 24-48 hours. Colonies that showed distinct morphological appearances were subsequently purified. Pure cultures were then subjected to various morphological and biochemical characterization tests to determine the identity of the bacteria isolates. Morphological test performed was Gram staining, while biochemical tests used in the identification of microorganisms were IMViC (Indole Methyl red Voges-Proskauer Citrate) and hemolysis tests.

DNA extraction and quantification

Bacteria cultures were enriched in Luria Broth medium at 37ºC for 24 hours and genomic DNA was extracted using Presto™ Mini gDNA Bacteria Kit (Geneaid, Shijr, TPE, TW). On the other hand, metagenome of 13 urine samples was prepared by using Geneius™ Micro gDNA Extraction Kit (Geneaid, Shijr, TPE, TW). Total concentration of DNA genome was quantified by using NanoDrop 2000 spectrophotometer (Thermo Scientific, Wilmington, USA).

PCR amplification of 16S rRNA gene

The genomic DNA of each selected bacterial isolates was amplified using PCR T1-thermocycler (Biometra, Goettingen, Germany) apparatus. Primer used to amplify 16S-rRNA gene were 63F (5'-CAGGCCTAACACATG CAAGTC-3') and 1387R (5'-GGG CGGWGTGTACAA GGC-3') (Marchesi et al. 1998). PCR amplification was performed in the total volume of 25 µL, each containing 12.5 µL *GoTaq Green Master Mix* 2x (Promega, Madison, WI, USA), 2.5 µL 63F and 1387R (10 pmol of each primer), ~100 ng of DNA template and 9 µL nuclease-free water. The PCR conditions were as follows: initial denaturation for 5 min at 94ºC and 35 cycles consisting of denaturation at 94ºC for 45 sec, annealing at 55ºC for 45 sec, 72ºC extension step for 1 min with post extension step at 72ºC for 7 min. The presence of DNA target band of expected size (1300 bp) was confirmed by 1% agarose gel electrophoresis.

Prior DGGE analysis, metagenome-derived urine samples were used as template from 16S-rRNA gene amplification by using primer pair of P338F-GC (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCAC GGGGGGACTCCTACGGGAGGCAGCAG-3') and P518R (5'-ATTACCGCGGCTGCTGG-3') (Overeas et.al 1997). Primer was set to fragment size of ±180 bp. The amplification was carried out using initial denaturing step of 5 minutes at 94ºC, followed by 35 cycles of 1 minute denaturing step at 94ºC, 30 seconds annealing at 55ºC, and 30 seconds extension at 72ºC, terminated by 3 minutes final extension at 72ºC.

DGGE analysis of the 16S rRNA gene

DGGE analysis was carried out using D-Code Universal Mutation Detection System (Bio-Rad, Hercules CA, US). As much as 25 µL (20 µL DNA + 5 µL Loading dye) PCR product was loaded in to vertical gel containing 8% (w/v) polyacrylamide gel (acrylamide-bis-acrylamide, 37:5:1). The denaturant gradient concentrations used were 30% and 70% (100% denaturant corresponded to 7 M urea and 40% (v/v) deionized formamide). The electrophoresis

Table 1. Biochemical characteristics of urine from health and UTI-diagnosed children

Sample	Age (years)	Gender (F/M)	Characteristics*								Status
			LEU (leu/µL)	NIT	URO (µmol/L)	PRO (g/L)	pH	KET (mmol/L)	SG	BIL (µmol/L)	
U1	6	F	70+	-	0.2(3.5)	30(0.3)+	5.0	160(16) ++++	1.030	-	Disease
U2	6	M	70+	-	0.2(3.5)	15(0.15)±	6.0	160(16) ++++	1.020	1(17)+	Disease
K1	6	M	-	-	0.2(3.5)	-	6.5	5(0.5)±	1.000	-	Health
U3	12	M	70+	-	0.2(3.5)	30(0.3)+	6.5	160(16) ++++	1.030	-	Disease
U4	10	M	70+	-	0.2(3.5)	-	7.5	-	1.005	-	Disease
U5	9	M	70+	-	0.2(3.5)	15(0.15)±	6.5	160(16) ++++	1.020	-	Disease
K2	10	M	-	-	0.2(3.5)	-	7.5	-	1.000	1(17)+	Health
U6	14	F	70+	-	0.2(3.5)	15(0.15)±	5.0	160(16) ++++	1.030	-	Disease
U7	17	F	15±	-	0.2(3.5)	300(3.0) +++	5.0	-	1.000	1(17)+	Disease
U8	17	M	70+	-	0.2(3.5)	15(0.15)±	6.0	160(16) ++++	1.015	-	Disease
U9	15	F	15±	-	0.2(3.5)	100(1.0) ++	7.0	-	1.000	1(17)+	Disease
U10	15	M	15±	-	0.2(3.5)	-	8.0	160(16) ++++	1.000	-	Disease
K3	15	F	-	-	0.2(3.5)	-	7.0	-	1.000	1(17)+	Health

*Notes : LEU : leukocytes; NIT : Nitrite; URO: Urobilinogen; PRO: Protein, KET: Ketone, SG: Specific Gravity, BIL: Bilirubin

process was carried out at a constant voltage of 150 V, 60ºC for 6 h in 1x TAE buffer (Tris-acetate-EDTA). After electrophoresis, the gel was stained by 0.1% Ethidium Bromide (EtBr) for 15 min, followed by rinsing with 300 mL of 1× TAE buffer before observing the band in G: BOX Gel Documentation (Syngene, Frederick, MD, US). DGGE bands were analyzed using CLIQS 1D software (Total Lab) to estimate the DNA total and volume band appeared on polyacrylamide gel. Clustering analysis was conducted based on CLIQS 1D analysis result. Each DGGE bands were excised using sterile scalpel and stored in microtube containing 100 µL nuclease-free water. The products of DGGE were incubated at 4ºC overnight. Each supernatant was used as the template for re-PCR process with the primers P338F (without GC-clamp) and P518R. The re-PCR condition was the same as previous PCR condition

16S rRNA gene sequencing for culturable and unculturable bacteria, bioinformatics analysis and phylogenetic tree construction

The PCR products of the 16S-rRNA gene of each genomic and metagenomic DNA were sequenced in a commercial company (First Base Co.). The sequencing of 16S rRNA gene was analyzed using Seqtrace 0.90 for both assembling and trimming process. Homology analysis was done by Basic Local Alignment Sequence Tools for Nucleotide (BLASTN) approach. Phylogenetic tree was constructed by using the neighbor-joining method with bootstrap replication of 2000× in MEGA 6.0 (Molecular Evolutionary Genetics Analysis 6.0) software (Tamura et al. 2013).

Statistical analysis of the DGGE profile was conducted by using software Paleontological Statistics (PAST3) (Hammer et al. 2001). Gel image of DGGE-band pattern was analyzed by using software CLIQS 1D. Clustering analysis was done by using binary data profile of the respective band pattern. Dendrogram was then constructed by using software UPGMA MEGA 7.0 .

Ethical considerations

The research protocol was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia, Jakarta (No.16-10-298).

RESULTS AND DISCUSSION

The diversity of urinary tract infection bacteria based on 16S rRNA gene

A total of 8 bacteria isolates were successfully isolated by using EMB Agar medium from 10 urine sample of UTI-diagnosed children treated at Indonesia Red Cross Hospital. The color of the isolates was varied within each isolate. Such distinct characters were also found in IMViC and hemolysis test (Table 2). Most of the isolates were Gram-negative which mostly showed α-hemolysin activity. Almost entire isolates showed negative results for indole test, except for SBU1, SBU2, and SBU4. Voges-Proskauer test only showed positive results for isolate SBU3 and SBU5. Methyl red test showed that 5 isolates showed positive results, and SBU3, SBU5, and SBU7 that show negative result. Meanwhile, Citrate test showed that only isolates SBU6 and SBU8 showed positive results.

The 16S rRNA genes from the genomic DNA of eight isolates were successfully amplified using 63F and 1387R primers (Figure 1). NCBI BLAST results of the cultured bacteria isolates SBU1, SBU2, SBU4 showed 98% with *Escherichia coli* strain CI5, *Escherichia coli* strain 275, *Escherichia coli* strain ST540 while SBU3 was 99% similar to *Acinetobacter calcoaceticus* strain NCTC7364, SBU5 was 98% similar to *Enterobacter cloacae* strain VRBG-62, SBU06 was 99% similar to *Citrobacter braakii* strain FDAARGOS 253, SBU7 was 97% similar to *Enterobacter asburiae* strain ATCC 35953 while SBU8 was 98% similarity to *Klebsiella pneumoniae* strain U25 (Table 3). Results from a phylogenetic analysis indicated that seven isolates belong to a cluster of the family Enterobacteriaceae and only 1 isolated belong to family of *Moraxellaceae* (Figure 2).

The diversity of bacteria based on metagenomic 16S rRNA-targeted PCR-DGGE analysis

The DGGE analysis of total bacterial community showed 16 different bands from 13 urine samples (Figure 3.A), According to DGGE 16S rRNA gene profile, total bacteria in urine of children with urinary tract infections were varied (Figure 3.A). However, only 9 bands were excised for further analysis. BLAST N analyses revealed that 9 bands were closely related with unculturable bacteria within 84%-96% of maximum identity (Table 4). However, there were 4 DGGE bands which were closely *Lactobacillus lactis* (Band 1), *Escherichia* sp. (Band 3), *Klebsiella* sp. (Band 4), and *Shigella flexneri* (Band 9). The phylogenetic tree showed that 9 bands were separated in to 2 clusters (Figure 4), band 1, 2, and 7 were cluster together (Cluster 1) and were closely related with *Lactobacillus* and Unculturable Bacterium. Other bands (band 3,5,6,8,9) formed a group (Cluster 2) that were closely related with *Escherichia*, *Klebsiella*, Unculturable bacterium and *Shigella*. Both cluster I and cluster II were separated from the outgroup (*Pyrolobus fumarii*). Clustering analysis was performed to assess genetic relatedness among the samples

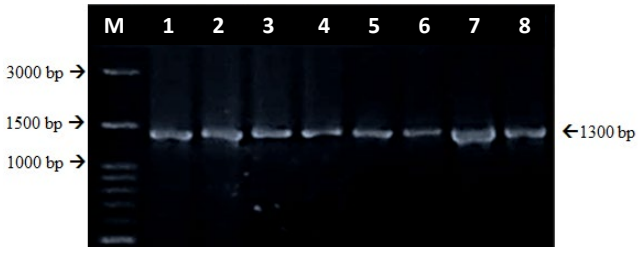


Figure 1. Photograph of PCR amplification products (amplicon) of 16S rRNA (1300 bp) using primer 63 and 1387R visualized on 1.5% agarose gel. Lanes from left to right: 100 bp marker (M), SBU1 (1), SBU2 (2), SBU3 (3), SBU4 (4), SBU5 (5), SBU6 (6), SBU7 (7), and SBU8 (8)

Table 2. The biochemical properties and characteristic of the isolates obtained from urine

Isolates	Color of colony	Shape	Gram	IMVIC Test				Hemolysin test
				MR	VP	Indole	Citrate	
SBU1	Green Metalic Sheen	Short Bacilli	-	+	-	+	-	α-hemolysin
SBU2	Green Metalic Sheen	Short Bacilli	-	+	-	+	-	α-hemolysin
SBU3	Dark purple	Coccobacilli	-	-	+	-	-	α-hemolysin
SBU4	Green Metalic Sheen	Short Bacilli	-	+	-	+	-	β-hemolysin
SBU5	Center dark purple dot	Short Bacilli	-	-	+	-	-	β-hemolysin
SBU6	Red pale	Coccobacilli	-	+	-	-	+	α-hemolysin
SBU7	Center dark purple dot	Bacilli	-	-	-	-	-	Ɣ-hemolysin
SBU8	Pink	Bacilli	-	+	-	-	+	Ɣ-hemolysin

Table 3. Results of BLAST analysis of the cultured bacteria based on 16S rRNA sequences

Isolates	Description	Query cover	E-Value	Identity	Accesion number
SBU1	*Esherichia coli* strain CI5	100%	0.0	98%	CP011018.1
SBU2	*Escherichia coli* strain 275	100%	0.0	98%	KX774265.1
SBU3	*Acinetobacter calcoaceticus* strain NCTC7364	99%	0.0	99%	LT605059.1
SBU4	*Escherichia coli* strain ST540	100%	0.0	98%	CP007391.1
SBU5	*Enterobacter cloacae* strain VRBG-62	100%	0.0	98%	KR265455.1
SBU6	*Citrobacter braakii* strain FDAARGOS 253	100%	0.0	99%	NR028894.1
SBU7	*Enterobacter asburiae* strain ATCC 35953	100%	0.0	98%	CP011863.1
SBU8	*Klebsiella pneumoniae* strain U25	100%	0.0	98%	CP013711.1

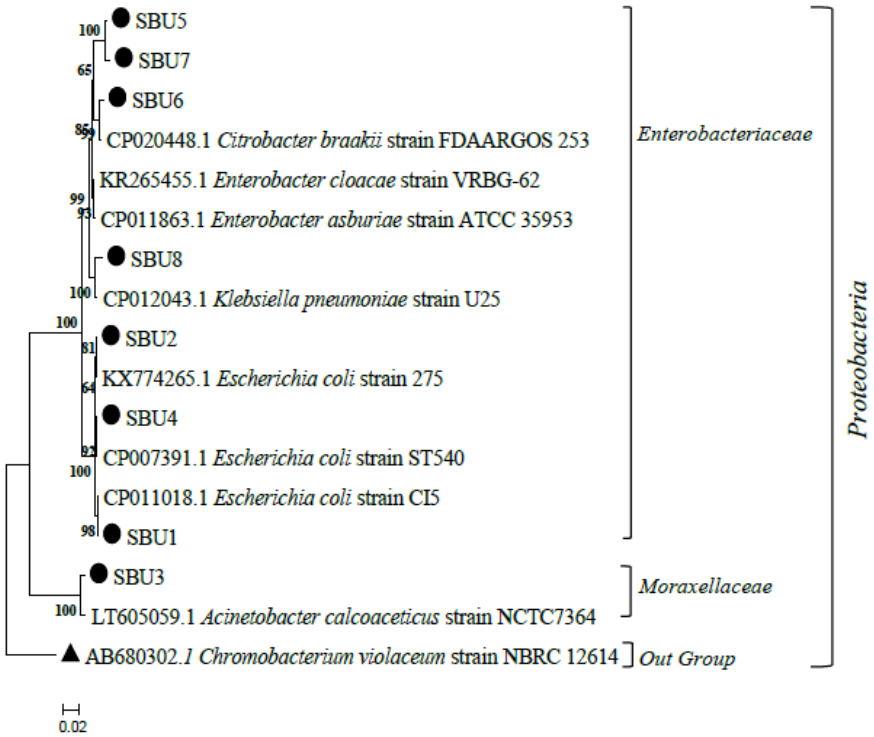


Figure 2. Phylogenetic tree of isolates bacteria from children urine diagnosed-UTI based on 16S rRNA genes sequences comparison. The tree was constructed from the isolated 16S rRNA bacterial sequences with their respective reference sequences from GenBank. This tree was analyzed by the neighbor-joining method in MEGA7 using *p-distance. Chromobacterium violaceum* was used as an out-group. Numbers at the nodes indicate the percentages of branch support of 2,000 bootstrap replicates. Bar 0.02 indicates nucleotides substitution per site

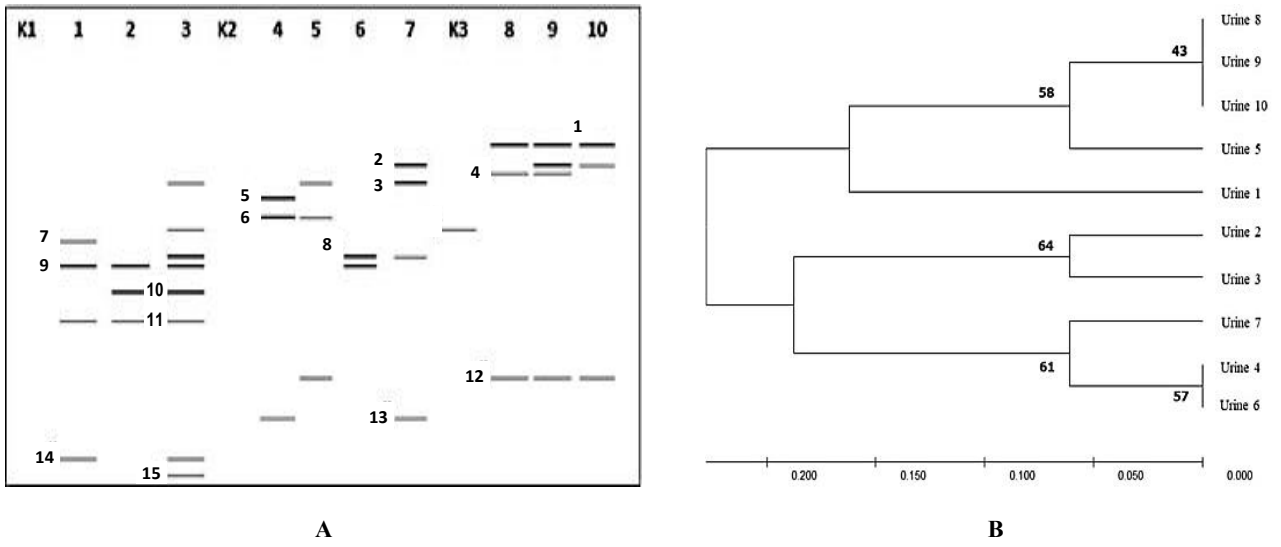
based on the banding pattern from DGGE profiles. Interestingly, community of bacteria within urine samples shares relatively similar diversity. In instance, urine 8, 9, and 10 were in one cluster, which means that the bacterial diversity holds high similarity. Likewise, urine 2-urine 3 and urine 4-urine 6 inhabited the same cluster (Figure 3.B). Another interesting observation was that diversity was present from urine sample with similar characteristic, i.e., urine 5 and 3 were found to be more different from each other. Similarly, urine 1 and urine 2 samples were also in a separate cluster. □

Discussion

The diversity of urinary tract infection bacteria based on 16S rRNA gene

UTI is the second most common site of bacterial infections in humans. Studies revealed that the causing bacterial agent of UTI infections belongs to the Enterobacteriaceae group (Prakash and Saxena 2013; Tarsali et al. 2013). This group of bacteria is known as one of human microbiome. The results of our study showed all isolates were Gram-negative bacteria, this was in accordance with the results of Dimitrov et al. (2004) and Abubakar (2009). The isolates had varied in hemolysis test and biochemical properties (Table 1) but morphologically similar based on biochemical characters. Most of the isolate showed common characteristics of the Enterobacteriaceae group Based on biochemical properties.

Molecular characterization using 16S rRNA sequences was indicated that seven isolates were included within Enterobacteriaceae and one isolate was within *Moraxellaceae*. Chaudhary and Murthy (2013) had reported that Enterobacteriaceae include species of *E. coli, K. pneumoniae, C. braakii, E. cloaceae* and *E. Asburiae* were the causal agents of UTI both in adult and children. We also obtained culturable *Acinetobacter* from the family of *Moraxelaceae* from the urine samples that were also found in the previous study by Sanjeev et al. (2013). Most of the bacteria observed in this study are expected as pathogenic organisms that cause urinary tract infection.



A **B**

Figure 3. A. DGGE illustration by CLIQS 1D Software. 1-16 bands were excised for further analysis. B. Dendrogram of clustering analysis of urinary tract infection bacteria, based on 16S rRNA gene. The scale below the dendrogram represents the evolutionary distance value. The numbers at each node are the bootstrap value of 1000 replicates. □

Table 4. Percentage of sequence similarity of 16S rRNA bacteria metagenome from urine of UTI-diagnosed children samples

Band	Description	Query cover	E-value	Identity	No. accession□
1	*Lactococcus lactic* subsp. Lactic strain CAU6764	86%	2e-59	94%	MF582669.1
2	Uncultured bacilli bacterium clone MS130A1A10	94%	1e-35	88%	EF699317.1
3	*Escherichia* sp. SP-3.0	68%	1e-52	95%	KX390660.1
4	*Klebsiella* sp. IPRI7	92%	1e-26	84%	KF478220.1
5	Uncultured bacterium clone nbw761c09c1	99%	5e-41	95%	GQ014562.1
6	Uncultured bacterium clone F1Q32TO03C8FJ3	54%	2e-09	95%	GU746413.1
7	Uncultured bacterium clone ncd2303f07c1	92%	1e-32	84%	JF197795.1
8	Uncultured bacterium clone F5K2Q4C04I7QT3	58%	3e-18	85%	GU912114.1
9	*Shigella flexneri* strain RSHD96	65%	5e-46	96%	MF326636.1

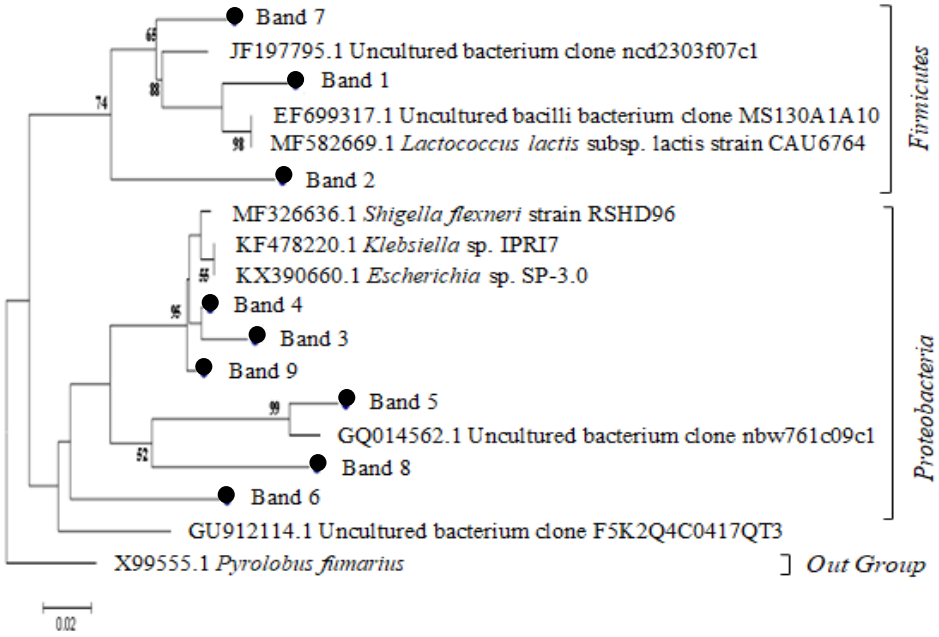


Figure 4 Phylogenetic tree of unculturable bacteria from children urine diagnosed-UTI based on 16S rRNA sequences. The tree was constructed from the isolated 16S rRNA bacterial sequences with their respective reference sequences from GeneBank. This tree was analyzed by the neighbor-joining method in MEGA7 using Tamura3-Parameter. *Pyrolobus fumari*i was used as an out-group. Numbers at the nodes indicate the percentages of branch support of 2,000 bootstrap replicates. Bar 0.02 indicates nucleotides substitution per site

We also observed the presence of bacteria in the urine of non-UTI samples, such as *Staphylococcus epidermidis*. Meanwhile, Enterobacteriaceae group such as *E.coli* that is a common cause of UTI was not detected in our study. *Staphylococcus epidermidis* is a mostly found as human skin microbiota but usually not regarded as the causative agent of UTI in children with no pre-existing conditions. Hagler and Dobkin (1990) stated that this organism was commonly associated with UTI's patient of indwelling urinary catheters and other instrumentation in the urinary tract. *Staphylococcus epidermidis* is considered as a contaminant when it is grown in healthy urine sample.

The diversity of bacteria based on metagenomic 16S rRNA-targeted PCR-DGGE analysis

The bacterial communities of 13 urine samples were analyzed by PCR-DGGE method. The presence of the DGGE band is used to describe the shape of community structure, diversity, and relatedness among the samples (Han et al. 2014). The results of our findings suggested that all urine samples diagnosed-UTI have different diversity compared to those of the non-UTI samples. Urine sample 3 (urine of diagnosed-UTI) showed as the highest bacterial diversity than the other urine samples. Such condition may likely facilitate the colonization of bacteria in the vaginal wall, rectum, urethra and move up into the ureter and bladder (Fouts et al. 2012). Population and microbial diversity can be affected by several factors such as gender, urine characteristic, and severity of illness suffered (Flores-Mireles et al. 2015).

Metagenomic analysis showed that urine sample is mostly colonized by bacteria identified as uncultured bacteria. Three bands were identified as *Proteobacteria* and the other one band was *Firmicutes*. The occurrence of these bacterial phyla has also been reported previously in various studies by using either culture-dependent methods (Liu et al. 2017). In our study, we observed bacterial isolates belong to *Gammaproteobacteria,* these included *Shigella flexneri*, *Klebsiella* sp., and *Escherichia* sp. Those bacterial isolates were found as members of the normal intestinal microbiota of humans and animals, and were reported to cause infection including urinary tract infection (Dhingra 2008; Nielseen et al. 2014). *Firmicutes* bacteria found in this study was *Lactococcus lactis*, that was commonly used in manufacturing dairy products and rarely considered as pathogenic in humans. However, these species cause significant clinical infections in immuno-compromised patients (Newby 2014). Some studies (Glikman et al. 2010; Topcu et al. 2011; Uchida et al. 2011) have reported that *Lactococcus* infected cases included brain abscess, catheter-related bacteremia, meningitis and septicemia, especially in children.

This is the first study to reveal the microbial diversity of bacteria in urine sample of UTI-diagnosed children in Indonesia. Most of the isolated bacteria were considered as pathogen, based on hemolysis assay. The diversity of bacteria comprises both cultured and uncultured bacteria. The combined culture and DGGE approaches provide an informative appraisal of the urinary tract infection bacteria diversity. According to phylogenetic analysis of culturable bacteria with 16S rRNA genes, that most of them are Enterobacteriaceae group, only one was closely related to *Moraxellaceae* group, however, all isolate identified as a group of *Proteobacteria*. In addition phylogenetic analysis

of DGGE based on 16S rRNA genes showed that most of them are uncultured bacteria, only four were identified as a group of *Proteobacteria* and *Firmicutes.*

ACKNOWLEDGEMENTS

The authors would like to thank the Indonesia Red Cross Hospital for support. We also thank contributors who collected samples used in this study, as well as patients and their families, whose help and participation made this study possible.

REFERENCES

Abubakar EM. 2009. Antimicrobial susceptibility pattern of pathogenic bacteria causing urinary tract infections at the specialist hospital, Yola, Adamawa state, Nigeria. J Clin Med Res 1: 001-008.

Chaudhary NK, Murthy SM. 2013. Urinary tract infection: etiology and antimicrobial resistance with reference to adhesive organelles. J Drug Delivery Therapeut 3: 93-98.

Dhingra KP. 2008. A case of complicated urinary tract infection: Klebsiella pneumonia emphysematous cystitis presenting as abdominal pain in the emergency department. West JEM 9: 171-173.

Dimitrov TS, Udo EE, Emara M, Awni F, Passadilla R. 2004. Etiology and antibiotic susceptibility patterns of community-acquired urinary tract infection in a Kuwait hospital. Med Princ Pract 13: 334-339.

Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. 2016. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 13: 269-284.

Fouts DE, Pieper R, Szpakowski S, Pohl H, Knoblach S, Suh MJ, Huang ST, Ljungberg I, Sprague BM, Lucas SK, Torralba M, Nelson KE, Groah SL. 2012. Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. J Transl Med 10: 174-191.

Giske CG, Gezelius L, Samuelsen, Warner M, Sundsfjord A, Woodford N. 2011. A sensitive and specific phenotypic assay for detection of metallo-β-lactamases and KPC in Klebsiella pneumoniae with the use of meropenem disks supplemented with aminophenyl boronic acid, dipicolinic acid and cloxacillin. Clin Microbiol Infect 17: 552-556.

Glikman D, Sprecher H, Chernokozinsky A, Weintraub Z. 2010. *Lactococcus lactis* catheter-related bacteremia in an infant. Eur J Pediatr 38: 145-146.

Hagler S, Dobkin D. 1990. Urinary tract infection in the male caused by Staphylococcus epidermidis associated with diverticulum of the bladder. Clin Pediatr 28: 527-528.

Hammer O, Harper DAT, Ryan PD. 2001. PASt: Paleontological Statistics software package for education and data analysis. Palaeontol Electron 1: 1-9.

Han PP, Shen SG, Jia SR, Wang HY, Zhong C, Tan ZL, Lv HX. 2014. Comparison of bacterial community structures of terrestrial cyanobacterium *Nostoc flagelliforme* in three different regions of China using PCR-DGGE Analysis. World J Microbiol Biotechnol 317: 1061-1069.

Hidayah N, Kusuma PA, Noormanto. 2011. Diagnostic tests of microscopic and urine dipstick examination in children with urinary tract infection. Paediatr Indonesia 51: 252-255

Kimura N. 2006. Metagenomics: access to unculturable microbes in the environment. Microbes Environ 21: 201-215.

Liu F, Zongxin L, Yonghong X, Longxian Lv, Qing Y, Baohong W, Haifeng L, Li Z, Ping J, Wei W, Lanjuan L. 2017. Dysbiosis of urinary microbiota is positively correlated with type 2 diabetes mellitus. Oncotarget 8: 3779-3810.

Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Hiom SJ, Wade WG. 1998. Design and evaluation of useful bacteria specific PCR primers that amplify genes coding for bacteria 16S rRNA. Appl Environ Microbiol 64: 795-799.

Minardi D, d'Anzeo G, Cantoro D, Conti A, Muzzonigro G. 2011 Urinary tract infections in women: etiology and treatment options. Intl J Gen Med 4: 333-343.

Mireles AL, WalkerJN, Caparon M, Hultgren SJ. 2015. Urinary tract infections; epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 13: 269-284.

Najar MS, Saldanha CL, Banday KL. 2009. Approach to urinary tract infection Indian. J Nephrol 19: 129-139.

Newby B. 2014. Urinary tract infection in a preterm neonatal caused by *Lactococcus lactis*. Can J Hosp Pharm 67: 453-454.

Nielsen KL, Pia D, Preben L, Niels FM. 2014. Faecal Escherichia coli from patients with *E. coli* urinary tract infection and healthy control who have never had a urinary tract infection. J Med Microbiol 63: 582-589.

Overeas L, Foney L, Daae FL, Torsvik V. 1997. Distribution of bacterioplankton in meromictic Lake Saelevannet, as determined by denaturing gradient gel electrophoresis of PCR amplified gene fragments coding for 16S rRNA. Appl Environ Microb 63: 3367-3373.

Prakash D, Saxena RS 2013 Antimicrobial susceptibility pattern of human pathogenic bacteria related to Enterobacteriaceae family causing urinary tract infection. Adv Appl Sci Res 4: 98-104.

Salvatore S, Salvatore S, Cattoni E, Siesto G, Serati M, Sorice P, Torella M. 2011. Urinary tract infection in woman. Eur J Obstet Gynecol Reprod Biol 156: 131-136.

Sanjeev H, Swathi N, Asha P, Rekha R, Vimal K, Ganesh HR. 2013. Systematic review of urinary tract infection caused by Acinetobacter species among hospitalized patients. Nitte Univ J Health Sci 3: 7-9.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. Mega 6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30: 2725-2729.

Tarsali GS, Stavros A, Matthew EF. 2013. Evaluation of antimicrobial susceptibility of Enterobacteriaceae causing urinary tract infection in Africa. Antimicrob Agents Chemother 57: 3628-3639

Topcu Y, Akıncı G, Bayram E, Hız S, Turkmen M. 2011. Brain abscess caused by *Lactococcus lactis* cremoris in a child. Eur J Pediatr 170: 1603-1605

Uchida Y, Morita H, Adachi S, Asano T, Taga T, Kondo N. 2011. Bacterial meningitis and septicemia of neonate due to *Lactococcus lactis*. Pediatr Intl 53: 119-120.

WHO. 2011. Infectious Disease. Department of Child and Adolescent Health and Development. World Health Organization, Geneva, Switzerland. http://www.who.int/topics/infectious_disease/en/.