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Anti-Microbial Peptides: A New Biomarker Of Urinary Tract Infection In Children.

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ABSTRACT

Antimicrobial peptides (AMPs) are the main action of innate immunity in urinary tract which has antibacterial activity. Our objective was to determine relation between urinary AMP levels and positive urine cultures in children with suspected urinary tract infection (UTI).This cross sectional study included 90 children with suspected UTI.Urine samples was taken to assess urinary AMPs ashuman α-defensin5 (HD5) and human neutrophil peptides 1–3 (HNP1-3).by ELIZA. Of 90 patients, there was 65 (72.2%) with positive urine culture and 25 (27.8%) with negative urine culture. Urinary HD5 and HNP1-3 levels were significantly increased in culture-positive than culture-negative. Urinary HD5 and HNP1-3 were 3.25 ng/mgCr and 1.19 ng/mgCr in culture-positive versus 0.38 ng/mgCr and 0.20 ng/mgCr in culture-negative, (P = .000) in both. Areas under the ROC curves for HD5 and HNP1-3 were 1.00 (95% CI, 1.00-1.00) and 0.98 (95% CI, 0.96-1.00), respectively in relation to urine culture. HD5 and HNP1-3 sensitivities and specificities using multiple test thresholds showed that HD5 threshold of 0.75 ng/mgCr had sensitivity 100% and specificity 96% and HNP1-3 threshold of 0.50 ng/mgCr had sensitivity 98.5% and specificity 92%. In conclusion urinary AMPs as HD-5 and HNP1-3 were increased significantlyin children with positive urine cultures .So, urinary AMPs levels are promising markers for early diagnosis and treatment of UTI.

Keywords: Urinary tract infection (UTI), Antimicrobial peptides (AMPs), human α-defensin5 (HD5) and human neutrophil peptides 1–3 (HNP1-3).

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INTRODUCTION

Urinary tract infection (UTI) is a common and serious infection that places a significant burden on children's health care cost and antibiotic exposure (1) .The diagnosis of UTI depends on the suspected symptoms, pyuria, and laboratoryinvestigations. Unfortunately, in childrenthe UTI symptoms are not specific, and culture results are not present at the time of initial presentation. So, most of physician's depend on fast results of urinary dipstick to start empirical antibiotic treatment for a suspected UTI until culture results appear (2). This may lead to unnecessary antibiotic treatment or delayed diagnosis becauselimited utilityof urinalysis dipstick for both leukocyte esterase (LE) and nitrite (3). Comprehensive pediatric researchand a useful meta-analysisfor detecting positive urine cultureusing urine dipstick have shownsuboptimal sensitivity and/or specificity andfurther tests are required, (4). In our study, we measuredHuman neutrophil peptides 1–3 (HNP1-3) and Human α-defensin 5 (HD5) which are two peptides of the α-defensins groupas new biomarkers for UTI in children (5).

Antimicrobial peptides (AMPs) have role in protecting against uropathogens (6). They are the main action of innate immunity in the urinary tract which has antibacterial activity through various mechanisms, includingcelllysis, inhibition of bacterial binding, and induction of other immune components. Preliminary studies had revealed an increase in the urinary levels of some AMPs as result of infections, such as Human neutrophil peptides 1–3 (HNP1-3) and Human α-defensin 5 (HD5) (7) .It was found that both urinary HD5 and HNP1-3 levelsmay act as a new markers for early positive urine culture detection in children (8).

Our aim was to determine that AMPs profile in urine are promising novel biomarker for early diagnosis and treatment of children with suspected urinary tract infection. We hypothesized that AMPs concentrations would be higher in children with positive urine culture which will improve diagnostic accuracy for UTI.

MATERIALS AND METHODS

Study Design and protocol

It is a cross-sectional descriptive study Written informed consent was obtained from all subjects or their parents after full discussion about the aim of the study.

Study Population

The study included (90) children with symptoms suspected UTI attend to Nephrology clinic, Almonira Children Hospital, Cairo University. The study was performed; from June 2018 till March 2019.This study is from a project no.110503 done at the National Research Centre, Cairo, Egypt .Inclusion criteria are completion of urine culture and adequate excess urine sample volume for analysis of bothHD5 and HNP1-3. Exclusion criteria include antibiotics treatment 7 days before urine sample. Urine concentrations of HD5 and HNP1-3 will be measured by enzyme-linked immunosorbent assay. Urine culture is the reference standard.

All the studied patients were subjected to:

Complete history taking including: age of patients, sex, weight, height, and BMI.With stress on symptoms suggested UTI as fever, abdominal pain, dysuria, Incontinence and hematuria.

Urine was collected by midstream clean catch, then centrifuged and stored at -80̊ C.

Clinical examination:

1. Patients and controls were subjected to complete physical examination.
2. Anthropometric measurements in the form of weight, height The weight and height of the participants were measured up to 0.01 kg and 0.1 cm using a Seca Scale Standing Balance and a Holtain Portable Anthropometer (Holtain Ltd, Crymych, Wales, UK)., Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared.

Laboratory investigation:

Midstream urine samples were collected under complete aseptic, sterile conditions in sterile urine cups. All Urine samples were subjected to complete urine analysis and urine cultures. All samples were centrifuged and the supernatant for each sample was collected for urinary creatinine measurement and then stored at minus 80ºC for urinary Human Neutrophil Peptide 1-3 and Human Defensin Alpha 5 detection. Nitrite and leucocyte esterase testing was done using Medi-Test Combi 10 kit (MACHEREY-NAGEL GmbH & Co. KG · Neumann-Neander-Str. 6–8 · 52355 Düren · Germany). Cultures was done using OXOID dehydrated culture media (Oxoid Limited, Wade Road, Basingstoke, Hampshire, RG24 8PW, United Kingdom) including Columbia Blood Agar Base; Code: CM0331; (prepared using 5% sterile defibrinated blood), MacConkey Agar; Code: CM0007; and CLED; Code: CM0301cultre media. For urine creatinine detection to standardize urine concentration , Jaffe reaction method was done using Erba Manheim Creatinine Assay Kit, done using ERBA Automated Chemistry Analyzer (Erba XL 300, ERBA Mannheim, Germany).For urinary Human Neutrophil Peptide 1-3 and Human Defensin Alpha 5 detection measurement, Bioassay Technology Laboratory ELISA kit (Cat. No E0341Hu and E2405Hu respectively) was used (Bioassay Technology Laboratory, Ningguo Road, Yangpu Dist. Shanghahai, China).

Statistical analysis

Data was analyzed using Statistical Package for Special Science software computer program version 15 (SPSS Inc., Chicago, Illinois, USA). Continuous variables were expressed as mean and stranded deviation .Categorical variables were expressed as number (n), percent (%) and were compared using the Chi square test or Fischer's Exact tests, as indicated. Continuous variables were compared using independent t- test. The associations between variables were assessed by Pearson correlation analysis. In order to measure the sensitivity and specificity of HD5 and HNP1-3, receiver operating characteristic (ROC) curve was generated and the area under the curve (AUC) was calculated. An AUC of 0.5 is no better than expected by chance, whereas a value of 1.0 signifies a perfect biomarker (9).Multivariate regression analysis was used to test the association between multiple quantitative and qualitative independent variables with the dependent variable. P value< 0.05 was considered statistically significant (9).

RESULTS

The study included 90 patients, they classified according to the results of urine culture into 65 (72.2%) with positive urine culture = patients, (33 males and 32 females) and 25 (27.8%) with negative urine culture (10males and 15females) controls. There is no significant difference was found in sex between studied groups (P = 0.2).

In positive urine cultures the most common organism was Escherichia coli, accounting for (n = 32/65) (49.2%), followed by Klebsiella pneumoniae (n = 20/65) (30.8%), Gram –vebacilli (n = 7/65) (10.8%), Enterobactria (n = 2), Gram +vecocci (n=2), Candida (n=1) and Coaulase +ve streptococci (n=1). We found that fever was the most common symptom of urinary tract infection accounted 44(67.7%) followed by abdominalpain40 (61.5%) and dysuria43 (66.1%) respectively.

Both nitrite and leukocyte esterase were positive accounted (16/65) 24.6% and (50/65) 76.9 %respectively in subjects with positive urine culture.

Table 1showed descriptive statistics of demographic and laboratory data of cases including: (minimum and maximum reading, median, mean and SD) of age, weight, height, BMI, HD 5 and HNP 1-3.The average age of the studied patients was (5.8±.3.0 years), and their average BMI was (16.41±2.34) kg/m2.

Table 2 showed descriptive, demographic and laboratory data of controls including: (minimum and maximum reading, median, mean and SD) of age, weight, height, BMI, HD 5 and HNP 1-3.

The average age of the studied patients was (7.06±.3.24 years), and their average BMI was (17.30±2.73) kg/m2.

Table 3showed a comparison between demographic and laboratory data of patients and controls included in the study, urinary levels of HD5 and HNP1-3 were increased significantly in positive culture urine compared to negative culture urine. Mean concentration of HD5was3.25 ng /mg creatinine (ng/mgCr) in culture-positive urine samples versus 0.38 ng/mgCr in culture-negative (P = .000). Mean concentration of HNP1-3 was 1.19 ng/mgCr in culture-positive urine samples versus 0.20 ng/mgCr in culture-negative urine samples (P = .000).

Table 4 demonstrated the correlation between different indices in the studied groups and showed that HD 5 had positive correlation with organism, HNP1-3 and pus count(r = .381; p = 0.000), (r = .797; p = 0.000) and (r = .585; p = 0.000) respectively. And HNP 1-3 had significant positive correlation with organism, HD 5 and pus count (r = .318; p = 0.002), (r = .797; p = 0.000) and (r = .664; p = 0.000) respectively.

Table 5 represented the multiple regression analysis of Human Defensin Alpha 5 (HD 5) as the dependent variable, showed that leucocyte esterase and Human Neutrophil Peptide 1-3 (HNP 1-3) added statistically significantly to the prediction,(B= .216, p = .012) and ,(B= .666, p = .000) respectively.

Table6 represented the multiple regression analysis of Human Neutrophil Peptide 1-3 (HNP 1-3) as the dependent variable, showed that pus count and Human Defensin Alpha 5 (HD 5) added statistically significantly to the prediction, ,(B= .336, p = .000) and ,(B= .655, p = .000) respectively.

Table 7 showed the (AUC) of HD5 and HNP1-3 using ROC curve were 1.00 (95% CI, 1.00-1.00) and 0.98 (95% CI, 0.96-1.00), respectively in in culture-positive and culture- negative urine samples.(Figure1)

Table 8 showed HD5 and HNP1-3 sensitivities and specificities using multiple test thresholds, values are done in relation to urine culture and showed that HD5 threshold of 0.75 ng/mgCr had sensitivity = 100% and specificity = 96%, and HNP1-3 threshold of 0.50 ng/mgCr had sensitivity = 98.5% and specificity = 92%, provided maximal sensitivity and specificity of each.

Table 9 represented the (AUC) of HD5 and HNP1-3 using ROC curve were .849 (95% CI, .760 -.937) and 0.829 (95% CI, .733- .925), respectively in relation to leukocyteesterase(Figure 2).

Table 10 demonstratedHD5 and HNP1-3 sensitivities and specificities using multiple test thresholds, values are done in relation to leucocyte esterase and showed that HD5 threshold of 0.75 ng/mgCr had sensitivity = 98% and specificity = 59%, and HNP1-3 threshold of 0.50 ng/mgCr had sensitivity = 96% and specificity = 57% .

Table 1: Descriptive statistics of demographic and laboratory data of patients.

	Minimum	Maximum	Mean	Standard Deviation	Median
Age(Years)	1.6	13	5.82	3.00	5.3
Weight (Kg)	7.5	35	19.96	6.81	20
Height (Cm)	78	139	108.76	16.47	108
BMI Kg/ M2	9.45	21.93	16.41	2.34	16.46
HD-5 ng/ml	1.1	7.2	3.25	1.52	3.1
HNP 1-3 ng/ml	0.4	2.7	1.19	0.49	1.1

(BMI): Body mass index, (HD-5): Human Defensin Alpha 5, (HNP1-3):Human Neutrophil Peptide 1-3

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Table 2: Descriptive statistics of demographic and laboratory data of controls.

	Minimum	Maximum	Mean	Standard Deviation	Median
Age(Years)	2	13	7.06	3.24	7
Weight (Kg)	12	38	23.6	7.62	25
Height (Cm)	82	145	115.6	16.94	118
BMI Kg/ M2	11.65	24.46	17.30	2.73	16.80
HD-5 ng/ml	0.23	0.86	0.38	0.15	0.35
HNP 1-3 ng/ml	0.03	0.9	0.20	0.18	0.13

(BMI): Body mass index, (HD-5): Human Defensin Alpha 5, (HNP1-3):Human Neutrophil Peptide 1-3

Table 3: Comparison between demographic and laboratory data of patients and controls included in the study as (mean±SD).

	PC	N	Mean	Std. Deviation	sig
age	1.00	65	5.8277	3.00143	.107
	2.00	25	7.0600	3.24782	
BMI	1.00	65	16.414	2.34327	.127
	2.00	25	17.304	2.73795	
HD-5	1.00	65	3.2543	1.52170	.000*
	2.00	25	.3860	.15229	
HNP (1-3)	1.00	65	1.1909	.49237	.000*
	2.00	25	.2080	.18870	

1=patients 2=controls* Significant

(BMI): Body mass index, (HD-5): Human Defensin Alpha 5, (HNP1-3): Human Neutrophil Peptide 1-3

Table 4: The results of correlation between the each index

	Organism		HD 5		HNP1-3		Pus count	
	r	p	r	p	r	p	r	p
Organism	1	.	.381	.000*	.318	.002*	.457	.000*
HD 5	.381	.000*	1	.	.797	.000*	.585	.000*
HNP1-3	.318	.002*	.797	.000*	1	.	.664	.000*
Pus count	.457	.000*	.585	.000*	.664	.000*	1	.

(HD-5): Human Defensin Alpha 5, (HNP1-3): Human Neutrophil Peptide 1-3

* Significant

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Table 5: the multiple regression analysis of Human Defensin Alpha 5 (HD 5) as the dependent variable.

		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
HD5	=dependant	B	Std. Error	Beta		
	(Constant)	1.002	.886		1.132	.261
	age	.009	.042	.015	.216	.829
	BMI	-.006	.053	-.008	-.108	.914
	nitrite	.118	.325	.025	.363	.718
	leucoest	.792	.309	.216	2.561	.012*
	Pus count	4.91E-006	.000	-.023	-.244	.808
	organism	.037	.059	.048	.634	.528
	HNP1-3	1.974	.257	.666	7.669	.000*

Dependent Variable: HD5* Significant
Predictors: (Constant) age, BMI, nitrite, leucocyte, pus, organism, HNP1-3
(HD-5): Human Defensin Alpha 5, (HNP1-3): Human Neutrophil Peptide 1-3

Table 6: The multiple regression analysis of Human Neutrophil Peptide 1-3 (HNP 1-3) as the dependent variable.

		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
HNP	dependant	B	Std. Error	Beta		
	(Constant)	.218	.298		.734	.465
	age	-.012	.014	-.059	-.840	.404
	BMI	-.012	.018	-.047	-.656	.514
	nitrite	.024	.109	.015	.221	.826
	leucoest	-.085	.107	-.069	-.790	.432
	Pus count	-1.02E-006	.000	.336	3.857	.000*
	organism	-.012	.020	-.045	-.601	.549
	HD5	.221	.029	.655	7.669	.000*

Dependent Variable: HNP1-3* Significant
Predictors: (Constant) age, BMI, nitrite, leucocyte, pus count, organism, HD 5

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Table 7: Area under the Curve (AUC) for urinary HD5 and HNP1-3

Test Result Variable(s)	AUC	Asymptotic 95% Confidence Interval	
HD5	1.000	1.000	1.000
HNP1-3	.985	.961	1.009

(HD-5): Human Defensin Alpha 5, (HNP1-3): Human Neutrophil Peptide 1-3

Table 8: Sensitivity and specificity of urinary HD5 and HNP1-3

Test Result Variable(s)	Value	sensitivity	specificity
HD5	0.75	100%	96%
HNP1-3	0.50	98.5%	92%

(HD-5): Human Defensin Alpha 5, (HNP1-3): Human Neutrophil Peptide 1-3

Table 9: Area under the Curve (AUC) for urinary HD5 and HNP1-3

Test Result Variable(s)	AUC	Asymptotic 95% Confidence Interval	
HD5	.849	.760	.937
HNP1-3	.829	.961	.925

(HD-5): Human Defensin Alpha 5, (HNP1-3): Human Neutrophil Peptide 1-3

Table 10: Sensitivity and specificity of urinary HD5 and HNP1-3

Test Result Variable(s)	Value	sensitivity	specificity
HD5	0.75	98%	59%
HNP1-3	0.37	96%	57%

(HD-5): Human Defensin Alpha 5, (HNP1-3): Human Neutrophil Peptide 1-3

Figure1: ROC curves for HD5 and HNP1-3 in culture-negative and culture-positive urine samples. The diagonal line represents a test with no diagnostic value.

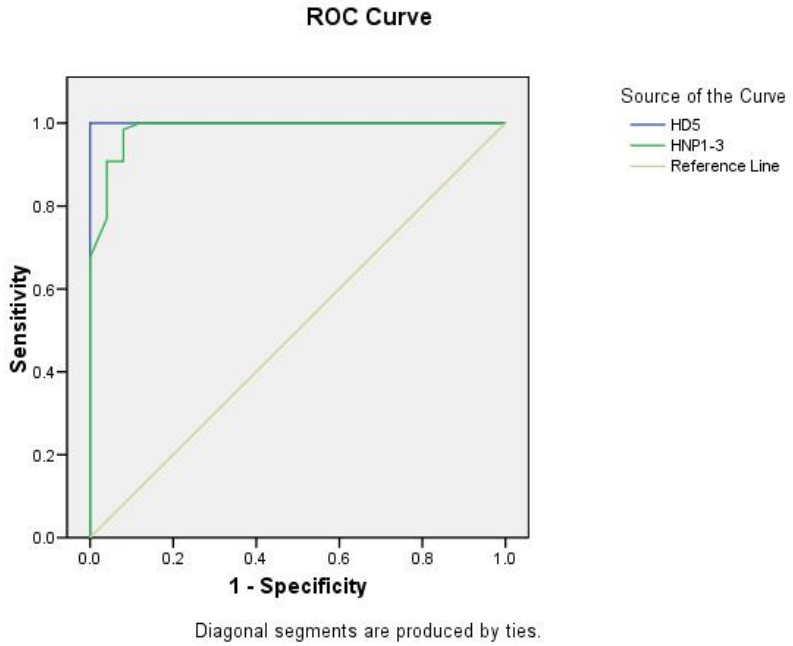

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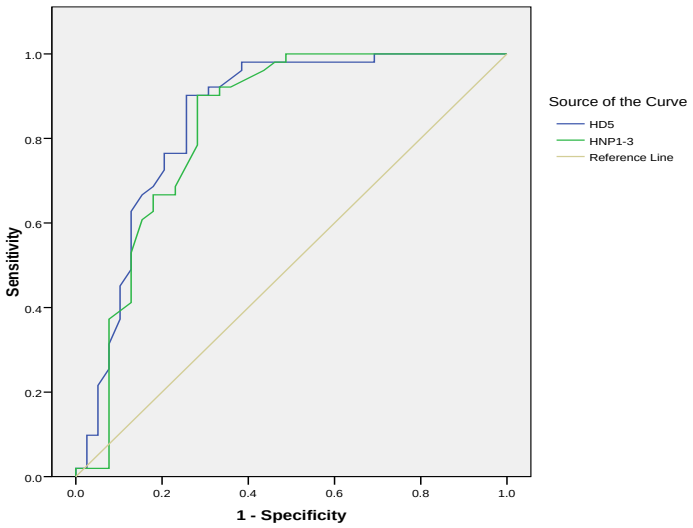
Figure 2: ROC curves for HD5 and HNP1-3 in relation to leukocyte esterase, the diagonal line represents a test with no diagnostic value.



ROC Curve

DISCUSSION

Our findings suggested that anti-microbial peptides might be good markers for positive urine culture. If confirmed, AMP concentrations could lead to accurate and more timely UTI diagnosis in children, leading to decreases in health care costs and unnecessary antibiotics use (10).

Both HD5 and HNP1-3 were formed during UTI.Our study showed increase concentrations of HD5 and HNP1-3 in positive culture than negative culture urine samples this agree with previous researches done in small numbers of children and adults (11, 12)., with areas under curves (AUC) of 1.000 and 0.98 for HD5 and HNP1-3, respectively, this agree with results of Watson et al, 2016 which show AUC of 0.86 and 0.88 for HD5 and HNP1-3, respectively using ROC curve. .In our study, urinary levels of HD5 increased eight-fold in children with positive urine culture than in culture negative children while Caterino et al, 2015 showed five times or more increase in HD 5 in culture positive than culture negative. Urinary HNP1-3 was five times or more great in positive urine culture than in culture negative this agree with results of (Caterino et al, .2015).The study showed no relation between ages, sex or body mass index in the studied group. Searching for recent biomarkers ofUTI was due to limitation of currently used (8).

This study showed significant correlation between HD5, HNP1-3, pus count and leucocyte esterase in studied subjects. This could be explained by the fact that patients hadalready positive urine cultures. The multiple regression analysis of Human Defensin Alpha 5 (HD 5) as the dependent variable, showed that leucocyte esterase and Human Neutrophil Peptide 1-3 (HNP 1-3) added statistically significantly to the prediction, p = .012 and p = .000 respectively, as bothHNP1-3 and LE are markers of neutrophil and thus explain pyuria(11). In multiple regression analysis of Human Neutrophil Peptide 1-3 (HNP 1-3) as the dependent variable, showed that pus count and Human Defensin Alpha 5 (HD 5) added statistically significantly to the prediction p = .000 in both as AMPs are produced by uroepithelial cells and may be produced when pathogens enter the urinary tract (5). The areas under the curve for HD5 and HNP1-3 using ROC analysis were 1.00 (95% CI, 1.00-1.00) and 0.98 (95% CI, 0.96-1.00), respectively in relation to culture of urine, but the AUC for HD5 and HNP1-3 using ROC curve were .849 (95% CI, .760 -.937) and 0.829 (95% CI, .733- .925), respectively regarding to leucocyte esterase and thus generally indicate “good” overall diagnostic value of these biomarkers (13), especially when referred to culture give best results than when referred to leucocyte estrae. 30% of both nitrites and (LE) reagent stripsare negative in the presence of positive culture, and in negative cultures more than 50% of strips are positive (14) this coincides with us as percent of nitrite positive in our patients were 24.6% and LE was 76.9%in spite of all patients had positive urine culture. Diagnostic uncertainty created when nitrite is negative and LE is positive and this is common (15) .So we need

rapid tests to allow diagnostic accuracy of UTI (Watson et al, .2016). In our study HD5 and HNP1-3sensitivities and specificities values are done in relation to urine culture, showed that HD5 threshold of 0.75 ng/mgCr had sensitivity = 100% and specificity = 96%, and HNP1-3 threshold of 0.37 ng/mgCr had sensitivity = 100% and specificity = 88%, provided maximal sensitivity and specificity of each, but Sensitivities and specificities of multiple test thresholds across the range in relation to leucocyte esterase of HD5 and HNP1-3 values showed that HD5 threshold of 0.75 ng/mgCr had sensitivity = 98% and specificity = 59%, and HNP1-3 threshold of 0.50 ng/mgCr had sensitivity = 96% and specificity = 57%, this higher sensitivity and specificity in relation to culture than to leucocyte estrase explained good prediction of UTI (8).

So that AMPs like HD5 and HNP1-3 could considered a promising diagnostic tests to improve UTI diagnostic accuracy.

Potential weaknesses in this study, is that we did not detect AMPs differences between cystitis and pyelonephritis .we take positive urine culture from subjects have symptoms of urinary tract infection, so it is not clear how asymptomatic bacteruria can affect AMPs.

CONCLUSIONS

HD5 and HNP1-3 levels in urine were increased significantly in children with positive urine cultures. So we conclude that urine AMP profiles are promising markers for early diagnosis and treatment of UTI.

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