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Prevalence of urinary tract infections and antibiotic susceptibility pattern of uropathogens isolated from children attending Brak general hospital and Sebha medical center in South Libya

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مدى انتشار التهابات المسالك البولية ونمط حساسية المضادات الحيوية لمسببات الأمراض البولية المعزولة من الأطفال المترددين على مستشفى براك العام ومركز سبها الطبي في جنوب ليبيا

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Abstract:
Urinary tract infection (UTI) is a significant cause of morbidity in children. Delayed treatment is associated with complications that may result in chronic kidney disease and, subsequently, end-stage kidney disease and it is also a significant health concern due to multidrug-resistant (MDR) organisms. Therefore, it is necessary to have a current understanding of the antibiotic susceptibility pattern of uropathogens to manage UTI effectively. This study aims to determine the prevalence and antibiotic susceptibility of uropathogens isolated from UTI children attending brak hospital and sebha hospital. Standard protocols for microbiological processes were followed in the collection and processing of catch midstream urine samples. Out of the 214 child samples, 63 (29.4%) samples had positive microbiological culture. Females had a higher prevalence of UTI (18.2%) than males (11.2%), The most susceptible age group was 5 months –5 years as compared other ages. The most prevalent pathogen identified were Escherichia coli (50.8%), followed by Klebsiella spp. (17.5%), Pseudomonas spp (14.3%). Proteus spp (11.1%) and Staphylococci spp. (6.3%). Our study's susceptibility pattern also showed that the most effective antibiotics against the isolated bacteria were imipenem and amikacin. However, the most resistant drugs were ampicillin and amoxicillin/clavulanic acid, while the other drugs affected the isolated bacteria differently. Children's UTI treatment necessitates particular attention to important factors like incidence rate, laboratory diagnosis, and antibiotic susceptibility testing because UTIs are among the most common bacterial illnesses. It also suggests that doctors should modify their treatment plan based on antimicrobial susceptibility results.

Keywords: Urinary Tract Infection (UTI), children, uropathogens, prevalence, antibiotic.

الملخص:
يُعدّ التهاب المسالك البولية سببًا رئيسيًا للإمرضية لدى الأطفال. ويرتبط تأخير العلاج بمضاعفات قد تؤدي إلى أمراض الكلى المزمنة، ثم الفشل الكلوي النهائي، كما يُشكّل مصدر قلق صحي كبير نظرًا لإنتشار الكائنات المقاومة للأدوية المتعددة. لذا، من الضروري فهم نمط حساسية مسببات التهاب المسالك البولية للمضادات الحيوية لإدارة هذا الإلتهاب بفعالية. تهدف هذه الدراسة إلى تحديد مدى إنتشار مسببات إلتهاب المسالك البولية المعزولة من عينات بول الأطفال المصابين بإلتهاب المسالك البولية في مستشفيي براك وسبها، ومدى حساسيتها للمضادات الحيوية. وقد تم إتباع البروتوكولات القياسية للعمليات الميكروبيولوجية في جمع ومعالجة عينات البول المأخوذة من منتصف مجرى البول. من بين 214 عينة من الأطفال، أظهرت 63 عينة (29.4%) نتائج إيجابية في الزرع الميكروبيولوجي. وكانت نسبة إنتشار إلتهاب المسالك البولية أعلى لدى الإناث (18.2%) مقارنةً بالذكور (11.2%). وكانت الفئة العمرية الأكثر عرضة للإصابة هي من 5 أشهر إلى 5 سنوات مقارنةً بالفئات العمرية الأخرى. كانت الإشريكية القولونية (50.8%) أكثر مسببات الأمراض شيوعًا، تليها الكلبسيلة (17.5%)، ثم الزائفة (14.3%)، والبروتيوس (11.1%)، وأخيرًا المكورات العنقودية (6.3%). وأظهر نمط حساسية المضادات الحيوية في دراستنا أن أكثر المضادات فعالية ضد البكتيريا المعزولة هي الإيميبينيم والأميكاسين. في المقابل، كانت الأمبيسيلين والأموكسيسيلين/حمض الكلافولانيك أكثر المضادات مقاومة، بينما أثرت المضادات الأخرى على البكتيريا المعزولة بشكل مختلف. يتأثر علاج إلتهابات المسالك البولية لدى الأطفال بعوامل مهمة مثل معدل الإصابة، والتشخيص المختبري، وإختبار حساسية المضادات الحيوية، لأن التهابات المسالك البولية من أكثر الأمراض البكتيرية شيوعًا. كما يشير ذلك إلى ضرورة تعديل الأطباء لخطة العلاج بناءً على نتائج اختبار حساسية المضادات الحيوية.

الكلمات المفتاحية: إلتهاب المسالك البولية، الأطفال، مسببات الأمراض البولية، الإنتشار، المضادات الحيوية.

Introduction:

Urinary tract infections (UTIs) are among the most common bacterial diseases in childhood [1-3] Urinary tract infections (UTIs) are a common reason for children to present to hospital emergency departments [4]. It is challenging to diagnose UTIs in children because the symptoms are frequently vague and insensitive, particularly in those who are too young to communicate verbally. Although urine culture is considered the standard for the diagnosis of UTIs, urine testing may be afected by false positive and false negative results. Sample collection is challenging and urine contamination in children is frequent and can cause false positive diagnoses or obscure true positive infections, resulting in inappropriate treatmen [5].

False negative results from previous antibiotic use and low bacterial counts can also affect urine testing [6]. Both the lower urinary tract (known to as cystitis) and the upper urinary tract (referred to as pyelonephritis) may be impacted by the infection. Unfortunately, based on clinical symptoms and signs, it may be difficult, if not impossible, to differentiate between cystitis and pyelonephritis, particularly in infants and young children [7-8]. Management of UTIs in children requires timely decisions that balance the risks of secondary bacteraemia and sepsis if appropriate treatment is delayed, and the potential side efects of those treatments, as well as the growing public health risks from antimicrobial resistance associated with indiscriminate treatment.

Urinary tract infections in the children population have been linked to several risk factors, such as age, gender, fever, constipation, uncircumcision, lack of toilet training, bladder instability, infrequent urination, prior antibiotic use, vesicoureteral reflux, and the presence of nitrates in urine [8–10]. It also turns out that a particular bacterial strain's propensity to colonize and then invade the urinary system is increased by the pathogen's virulence factors. These factors include α-hemolysin, M hemagglutinin, endotoxin, cytotoxic necrotizing factor 1, K capsular antigen, a rigid cell wall, serum resistance ability due to the outer membrane protein TraT, aerobactin which supports growth by chelating iron, and adhesive capacity [11,12]. The three different types of adhesins identified on uropathogenic *E. coli* include type 1 pili (or fimbriae), Pfimbriae and X-adhesins [11,13].

Despite the flushing effect of urine flow, these adhesins help the bacteria cling to mucosal receptors in the uroepithelium. An intracellular biofilm forms after the uroepithelium is invaded, and this biofilm can shield the uropathogenic *E. coli* from the host immune system. [14,15]. Prompt diagnosis and appropriate treatment are essential to reduce the morbidity and consequences of UTI. However, the diagnosis of UTI in young children is often confused with nonspecific signs and symptoms of UTI [16,17]. Numerous studies demonstrate that oral and intravenous antibiotics are equally effective in treating UTIs [18]. However, research from wealthy nations indicates that bacteria that cause UTIs are becoming more resistant to popular antibiotics like trimethoprim-sulfamethoxazole. [19,20].

Gram-negative organisms highly contribute to the proportion of uropathogens isolated from children with UTI [21]. The most documented uropathogen is *Escherichia coli* (*E. coli*), which causes more than 80% to 90% of UTIs in neonate patients. This is since *E. coli* strains have attachment factors for the transitional epithelium of the bladder and ureters [22, 23]. *Klebsiella pneumoniae*, *Proteus mirabilis*, *Citrobacter*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, Enterococcus species, and *Serratia* species are among the other uropathogens that are frequently isolated in UTIs [24]. Boys are more likely than girls to have Proteus mirabilis [25]. Newborns are frequently the source of *Streptococcus agalactiae* isolates [26]. 15% of UTI cases are caused by *Staphylococcus saprophyticus*, which is identified in sexually active female teenagers [27].

Public health is seriously threatened by the growing prevalence of antibiotic resistance in uropathogens and UTI recurrence rates, which likely significantly increase the economic burden associated with these infections [23]. Identification of the organism and its and antibiotic susceptibility is crucial for managing UTI. The purpose of this study was to ascertain the frequency of UTIs and the testing of uropathogens for antibiotic susceptibility in children who were attending Brak General Hospital and Sebha Medical Center.

Methods:

Study design:

This was a study enrolling symptomatic and asymptomatic children attending Brak general hospital and Sebha medical center in South Libya. The inclusion criteria were age range 5 months to 15 years. Convenience sampling was used. The exclusion criteria were children who had recently taken antibiotics.

Sample collection:

The commonly advised urine sampling technique for children, in which parents are directed to obtain a urine sample under sterile settings, was used to obtain midstream urine samples from the children [28]. Thus, the lid was closed once the container was one-third full, and clean catch mid-stream urine samples were sent right away to the microbiology lab for examination in sterile containers. A cold box with ice blocks was used to keep the temperature between 4 and 8°C throughout transit to inhibit the growth of microorganisms [29].

Microscopic Examination of Samples:

Ten millilitres (10 ml) of sample was centrifuged at 2000 rpm for 5 minutes and the sediments mounted on a clean slide and observed under a microscope using a ×40 objective. Samples were observed for the presence of bacteria and other pathological indicators like crystals and epithelial cells [30]. Microscopy which revealed at least 5 white blood cells per high power field (HPF) and/or the presence of bacterial cells per HPF was suggestive of UTI [31].

Isolation and Identification of Bacteria:

Samples were thoroughly mixed and cultured as described by Ibeneme et al. [31]. Sample was inoculated onto Cysteine Lactose Elec-trolyte Deficient (CLED) agar and incubated aerobically at 37°C for 24 hours after which cultures were observed for growth. Pure isolates of the bacterial pathogen were preliminarily characterized by colony morphology then biochemical tests. The tests carried out were Gram staining, oxidase test, catalase test, coagulase test, growth on Mannitol Salt Agar (for Gram-positive cocci), and Triple Sugar Iron Agar for primary identification. The API 20E kit was used to confirm the identity of Enterobacteriaceae.

Antimicrobial Susceptibility Testing:

The identified uropathogens were subcultured two times before being used for antibiotic susceptibility tests. Antibiotic susceptibility test was carried out by Kirby Bauer disc diffusion method as per Clinical Laboratory Standards Institute (CLSI) guidelines [32] on Mueller Hinton agar. A bacterial suspension of each isolate was prepared in 0.5 mL of nutrient broth medium and the turbidity was adjusted to match that of 0.5 McFarland standards to obtain approximately the organism number of

1×10^6 colony forming units (CFU) per ml. A sterile swab was dipped into the suspension and the excess material was removed by pressing it against the sides of the tube. Then, the swab was applied to the center of the Mueller Hinton agar plate and evenly spread on the medium. Antibiotic discs were placed within 15 min of inoculation to Mueller Hinton agar seeded with each isolate and were incubated for 24h at 37°C. Interpretation of antibiotic results was done by measuring the diameter of the zone of inhibition using a ruler according to the Clinical and Laboratory Standards Institute (CLSI) guideline [32] as susceptible (S), intermediate (I), or resistant (R). The following antibiotics were used: Ampicillin (AMP), Amoxicillin/clavulanic acid (AMC), Tetracycline (TE), Ciprofloxacin, (CIP), Cefixime (CFM), Gentamicin (GEN), Vancomycin (VAN), Amikacin (AMK), Imipenem (IMP).

Results:

214 children attending Brak general hospital and Sebha medical center between age group of 5 months to 15 years were enrolled for this study, of which 106 (49.5%) were female and 108 (50.5%) were male. However, only 63 (29.4%) of the total samples were positive for bacterial growth, while the other 151 (70.6%) samples had shown no bacterial growth. The UTI cases in female children were 39 (18.2%) while in male children was 24 (11.2%) (Table1).

Table (1): Urinary tract infection (UTI) status per gender, age.

Variables	Positive N (%)	Negative N (%)	Total N (%)
Gender			
Males	24 (11.2%)	84 (39.3%)	108 (50.5)
Females	39 (18.2%)	67(31.3%)	106 (49.5)
Age group (From 5 months)	Yes N (%)	No N (%)	
5 months - 5 years	32 (15%)	51 (23.8%)	83 (38.8)
6 years -10 years	18 (8.4%)	53 (24.8%)	71 (33.2)
11 years -15 years	13 (6%)	47 (22%)	60 (28)

Among the isolated uropathogens, *Escherichia coli* was common bacteria accounting for 50.8% of all isolates. The rate of other uropathogens isolated was *Klebsiella spp*. (17.5%), *Pseudomonas spp* (14.3%). *Proteus spp* (11.1%) and *Staphylococci spp*. (6.3%) (Table 2).

Table (2): Bacterial agents isolated form urine specimens.

Isolated organism	No. of children with UTI	Percentage (%)
E. coli	32	50.8
Klebsiella Spp	11	17.5
Pseudomonas Spp	09	14.3
Proteus Spp	07	11.1
Staphylococci Spp	04	6.3
Total	63	100

The results of susceptibility test showed that *E. coli* isolates were highly sensitive to imipenem (100%) followed by gentamicin and vancomycin (81.4%), then amikacin (68.8%). The sensitivity to cefixime, ciprofloxacin and tetracycline was low (46.9%,43.8% and 37.5%) respectively. Whereas these isolates showed high resistance to ampicillin (100%) and amoxicillin/clavulanic acid (85%). *Klebsiella spp* the second most isolated bacterium were highly sensitive to imipenem (91%), gentamicin (90.9%), amikacin (81.8%), vancomycin (72.7%) and tetracycline (54.5%), but highly resistant to ampicillin (81.8%) followed by amoxicillin/clavulanic acid (63.6%), ciprofloxacin (63.6%), and cefixime (54.5%).

The susceptibility of *Proteus spp* isolates showed that sensitivity rate to imipenem in the range of (100%) followed by gentamicin, vancomycin (85.7%), amikacin (71.4%), but all (100%) isolates were resistant to ampicillin, cefixime and ciprofloxacin followed by tetracycline and amoxicillin/clavulanic acid (85.7%). *Pseudomonas spp*. isolates showed low sensitivity to most tested antibiotics. The isolates were (77.7%) sensitive to imipenem, amikacin and (66.7%) to vancomycin, gentamicin, but they were highly (66.7-100%) resistant to the rest of tested antibiotics. The *Staphylococci Spp* showed 100%

sensitivity to imipenem and cefixime followed by amikacin, tetracycline 75% and ciprofloxacin, vancomycin, gentamicin it was 50%, whereas a high level of resistance was observed to ampicillin and amoxicillin/clavulanic acid (75%).

Table (3): Antimicrobial Susceptibility 0f isoleted Bacteria.

Tested antibiotics	**E. coli n=32**		**K. pneumoniae n=11**		**P. aeruginosa n=9**		***Proteus spp* n=07**		**Staphylococci Spp n =04**	
	no (%) S	no (%) R	no (%) S	no (%) R	no (%) S	no (%) R	no (%) S	no (%) R	no (%) S	no (%) R
IMP	32 (100%)	0	10 (91%)	1 (9%)	7 (77.8%)	2 (22.2%)	7 (100%)	0	4 (100%)	0
GEN	26 (81.2%	6 (18.8)	10 (90.9%)	1 (9.1%)	6 (66.7%)	3 (33.3%)	6 (85.7%)	1 (14.3%)	2 (50%)	2 (50%)
VAN	26 (81.3%	6 (18.8)	8 (72.7%)	3 (27.3%)	6 (66.7%)	3 (33.3%)	6 (85.7)	1 (14.3%)	2 (50%)	2 (50%)
AMK	22 (68.8%)	10 (31.2)	9 (81.8%)	2 (18.2%)	7 (77.7%)	2 (22.2%)	5 (71.4%)	2 (28.6%)	3 (75%)	1 (25%)
CFM	15 (46.9%)	17 (53.1%)	5 (45.5%)	6 (54.5%)	2 (22.2%)	7 (77.7%)	0	7 (100%)	4 (100%)	0
CIP	14 (43.8%)	18 (56.2%)	4 (36.4%)	7 (63.6%)	2 (22.2%)	7 (77.7%)	0	7 (100%)	2 (50%)	2 (50%)
TE	12 (37.5%)	20 (62.5%)	6 (54.5%)	5 (45.5)	3 (33.3%)	6 (66.7%)	1 (14.3%)	6 (85.7%)	3 (75%)	25%
AMP	0	32 (100%)	2 (18.2%)	9 (81.8%)	1 (11.1%)	8 (88.9%)	0	7 (100%)	1 (25%)	3 (75%)
AMC	2 (6.3%)	30 (93.7%)	4 (36.4%	7 (63.6%)	0	9 (100%)	1 (14.3%)	6 (85.7%)	1 (25%)	3 (75%)

Values in n indicates- Total no. of bacteria, no (%) S - Number and percentage of Sensitive bacteria, no (%) R Number and percentage of Resistance bacteria, Abbreviations: AMC, amoxicillin/clavulanic acid; CIP, ciprofloxacin; VAN, Vancomycin; AMP, ampicillin; AMK, amikacin; GEN, gentamicin; TE, tetracycline; IMP, imipenem; CFM: cefixime

Discussion:

Urinary tract pathogens, their physiological functions, and antibiotic susceptibility patterns have changed over time and place, and this will continue to change in the future. [33]. Therefore, the management of urinary tract infections depends on determining the organism and its susceptibility to antibiotics. This emphasizes how crucial it is for the doctor and the microbiologist to work closely together [34]. This study was conducted to determine the prevalence of UTIs, and to determine the drug susceptibility of bacterial uropathogens among children attending Brak general hospital and Sebha medical center.

A total of 214 study participants were enrolled in the present study, of whom,108 (50.5%) were females and 106 (49.5) were males. The ages of study subjects ranged from 5 months to 15 years. only 63 (29.4%) had urine samples with significant bacteriuria. This prevalence rate is higher as compared to previous studies, which account for 15.43% in Libya [35], Iraq 14.7%, [36], Ethiopia 15.8% [37], 21.6% Ethiopia [38], Tanzania 16.4% [39]. According to several studies, the frequency of UTIs is higher in females than in males [9, 40, 41,42] also, in the present study revealed a higher incidence of urinary tract infection in females than in males. Of the 106 females, 39 (18.2%) came up with positive cultures, while 24 (11.2%) of the 108 males had significant bacteriuria indicating that female patients were more affected than male patients. Among the culture positive study subjects, 32 (15%) of them were below 5 years of age. Our result in this regard was comparable to that reported in Libya [35].

Inappropriate toilet habits, a shorter urethra, and the proximity of the urethral meatus to the anus may all contribute to a higher incidence of UTI in women than in men. [40,41], and the high incidence of UTIs in children between the ages of 5 months and 5 years may be due to the overuse or unhygienic use of diapers and improper cleaning of the urogenital area. Therefore, personal hygiene and parental health education play an important role in preventing UTIs in both girls and boys. Sixty-three bacterial isolates were recovered from children attending Brak general hospital and Sebha medical center in this study. They were *Escherichia coli*, *Klebsiella spp*, *Pseudomonas spp*, *Proteus spp* and *Staphylococci Spp*. *Escherichia coli* was the most common organism isolated from children with significant bacteriuria and was isolated from 32 cases (50.8%) followed by *Klebsiella spp* from 11 cases (17.5%), *Pseudomonas spp* from 9 cases (14.3%), *Proteus spp* from 7 cases (11.1%), and *Staphylococci Spp* from 4 case (6.3%).

The frequency of bacterial isolates obtained in this study were comparable with different study findings done in different countries. For example, a study conducted in Libya [42] showed that, among bacterial isolates, 47.8% accounted for *E. coli*, and this was followed by *Klebsiella spp*. (22.1%), *Proteus spp*. (9.9%), *Pseudomonas spp*. (3.2%) and *Staphylococci Spp*. (12.2%). A study conducted in Nigeria [43] indicated that among all the bacteria isolated in their study, *E. coli* was the predominant organism and was responsible for (28.9%) of the cases of UTI, and this was followed by *Klebsiella spp*. (21.1), *Pseudomonas spp*. (13.2), *Proteus spp*. (7.9), *Staphylococcus spp* (5.3). Similarly, a study conducted in Tanzania [39] indicated that, Among the isolated uropathogens *E. coli* were common bacteria accounting for 65.7% of all isolates followed by *Klebsiella spp*. (17.1%), *Pseudomonas spp*, *Proteus spp* (11.4%) and *Staphylococci spp* (2.9%). Interestingly, most of the microbes found during this study are members of the Enterococcus genus, which inhabits the vagina, rectum, urinary system, and the area surrounding the bladder. When these bacteria get into the sterile urinary tract, they grow and cause infection [26].

In this study all isolated bacterial species were exposed to a range of antibiotics, nine antibiotics were used to study the antibiotic susceptibility of uropathogens isolated from urine samples in children under fifteen years old (Table 3). Isolates from urinary tract infections included in the current study showed varying levels of antibiotic susceptibility. *E. Coli* isolates were found to be highly sensitive to imipenem, Vancomycin, gentamicin but also sensitive with variable degree to amikacin this is consistent with the findings of the study by Mahmoud et al, in the city of Messalata, Libya [44] and less sensitive to cefixime and ciprofloxacin, however, the resistance was mostly toward Ampicillin and amoxicillin/clavulanic acid which is consistent with other studies [38,39]. These resistance levels are much higher than reported in previous studies [35, 45], tetracycline. *Klebsiella spp* isolates were found to be highly sensitive to, imipenem, gentamicin, Vancomycin and amikacin, and showed low sensitivity to cefixime ciprofloxacin but highly resistance to, ampicillin and amoxicillin/clavulanic acid.

The susceptibility of *Proteus spp* and *Pseudomonas spp* isolates showed sensitivity to imipenem, gentamicin. These results were consistent with prior research conducted in Libya and [42,46], but all approximately (100%) isolates were resistance to the rest of tested antibiotic. The present study also showed that 100% of *Staphylococci spp* isolates were sensitive to imipenem, cefixime. A sensitivity rate of 100% for this antibiotic has been observed by Prakash & Saxena, 2013 [47]. Also 100% of *Staphylococci spp* showed sensitivity to amikacin. High activity of amikacin agreed with another study that reported 71.6% of *Staphylococci spp* were sensitive [48], but a higher sensitivity rate (80%) has been reported earlier [49]. *Staphylococci spp* isolates in this study were highly (75%) resistant to ampicillin and amoxicillin-clavulanic acid. The same finding was reported in other studies [50]. Ampicillin and amoxicillin/clavulanic acid showed the highest level of antibiotic resistance among the isolated species, according to the results of the sensitivity test conducted on the bacteria.

The β-lactamase enzyme, which targets the β-lactam ring in these antibiotics' structures and gives them their activity, is most likely produced by these isolates. Consequently, while treating UTIs, these antibiotics ought to be used with a β-lactamase inhibitor, a resistance buster [51]. Another important reasons is that the drug quality, changing of uropathogens susceptibility results due to variance in bacterial strains, and differences in study area, right drug dosage, and therapy duration, Ease of access to medicines, whether from private or government pharmacies, especially in developing countries such as Libya, all these factors collectively or individually can lead to the emergence of such high resistant rates among uropathogens to these drugs which resulted in difficulty in the treatment of UTIs.

Conclusion and Recommendation

This study determined the prevalence of urinary tract infection in children and highlighted the major bacterial uropathogens involved in UTI and their antibiotic susceptibility. Furthermore, bacterial pathogen species and their frequency was slightly higher than previous studies in Libya, this study confirmed that the *E. coli*, *Klebsiella spp*, *Proteus spp*, *Pseudomonas spp*, and *Staphylococci spp* are the major uropathogens usually associated with UTI. Results also demonstrated that females are more susceptible to UTI than males. Additionally, imipenem and amikacin showed the highest activity against isolated bacteria, and they could be the drugs of choice for the treatment of UTI. On the contrary, ampicillin and amoxicillin/clavulanic acid should be excluded for UTI treatment in study area due to a very high resistance rate. This study also noted that most bacterial isolates exhibit varying resistance to multiple drugs. Therefore, it is recommended that appropriate antimicrobials be administered to reduce the risk of developing multidrug-resistant bacteria and avoid antibiotic ineffectiveness. A comprehensive screening program for children urinary tract infections should be implemented to accurately determine their prevalence and measure the resistance rate of urinary tract pathogens to common antibiotics.

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