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Research Article:

Bacterial Profile and Antibiotic Susceptibility Pattern of Uropathogens Causing Urinary Tract Infections in Quetta City, Pakistan

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Article Information

Abstract

Background: Irrational use of antibiotics and antibiotic resistance against uropathogens is a global catastrophe. Consequently, it is necessary to identify the antibiotic susceptibility pattern of uropathogens while managing Urinary Tract Infections (UTIs). Also, recognizing the susceptibility pattern of the uropathogens is essential for empirical antibiotic therapy. The current study aims to establish the bacterial profile and the antibiotic susceptibility pattern isolated from UTI patients in the northern Balochistan part of Pakistan. **Methods:** The study was carried out between January 2022 and December 2022. Clean catch midstream urine samples were collected. The isolation and antibiotic susceptibility procedures were performed through standard guidelines for microbiological techniques. One thousand forty-eight samples were collected and produced positive bacterial culture. The SPSS v 26 was used for data coding and descriptive statistics were applied accordingly. **Results:** Four hundred and forty patients (72%) were between 28 -37 years old; males contribute to (61.3%) of all samples. Microbiological cultures were found in 1048 patients with Escherichia coli being the dominant pathogen (1018, 97.1%) followed by Pseudomonas aeruginosa (202, 19.3%). The susceptibility pattern of our study revealed that Cefixime, Norfloxacin, and Nalidixic acid were the antibiotics with the highest resistance rates in bacterial uropathogens, at 100%, 94.0% and 93.7%, respectively. Meropenem, Amikacin, Tazobactam, Fosfomycin and Vancomycin were antibiotics with sensitivity rates exceeding 90%. **Conclusion:** The bacterial isolates identified in the current study reported high resistance to Cefixime, Norfloxacin and Nalidixic acid (predominantly for Escherichia Coli). This leaves clinicians with very limited options of drugs for the treatment of UTIs. Increased resistance to commonly prescribed antibiotics in UTIs warrants strict surveillance and monitoring of antibiotic use in the community.

1. Introduction

Urinary tract infections (UTIs) are a serious public health concern and a global increasing trend of the disease burden was observed between 1991 – 2019 (1). Caused by a variety of bacteria, the most frequent of which is Escherichia coli (2), nearly two hundred and fifty thousand people died of UTIs in 2019 contributing to 5.2 million Disability-Adjusted Life Years (3). Affecting men and women of all ages, UTIs are more prevalent in women owing to anatomical predisposition or a high bacterial load in the urothelial mucosa, as well as other host variables such as urinary obstruction, sexual activity, and pregnancy (4). Today, the novel models emerging in recent years have allowed researchers to examine both uncomplicated and complicated UTIs extensively. Summarizing, the very models suggest that a healthy bladder is not always sterile, and a picture of the urine microbiome develops with time. These advancements have the potential to provide further light on the molecular processes of virulence in UTIs and must be considered while managing UTIs (5).

Shifting our concerns to the management of UTIs, uncomplicated cases do not necessarily require a urine

culture. However, recurrent UTIs or patients experiencing unusual symptoms, such as obstruction or the persistence of haematuria following infection suppression warrant immediate assessment and urine culture. Nonetheless, it is a practice of the developing world to identify UITs on apparent complaints of the patients (6,7). Supporting our prerogative, data from local microbiological facilities also favour the experiential selection of antibiotics for UTI-related management, and in very rare cases the specimen is sent to laboratories for culture and sensitivity assessment (8). Consequently, UTIs have been empirically managed which resulted in the development of antibiotic misuse and resistance (9). We must remember that this increased frequency of resistance is now a significant public health issue and susceptibility screening is critical for producing up-to-date epidemiological data for healthcare professionals and policymakers (10,11).

Within this context, the resistance profile of UTIs among diverse geographical regions is poorly explored and least reported in the literature. The same is the case with the Baluchistan province of Pakistan which is rich in antibiotic resistance and both multi-drug resistant and extensively drug-resistant bacteria are identified in the last few years (12-14).For example, resistance to quinolones has increased considerably and the 2016 outbreak of Salmonella was 100% resistant to fluoroquinolones (15).Similarly, The Institute for Health Metrics and Evaluation warned about five pathogens to be aware of in Pakistan including *Escherichia coli* which resulted in 31,300 unnecessary resistant-related deaths in 2019 (16). Correlating the increased antibiotic resistance in Pakistan, infectious diseases are regularly treated empirically as microbiological facilities are either inaccessible or absurdly expensive for most of the population (12,17). Consequently, we aimed to identify the bacterial profile and antibiotic susceptibility patterns of uropathogens causing urinary tract infections in Quetta City, Pakistan.

2. Methods

2.1 Study design, settings, and sampling

A hospital-based, cross-sectional study was conducted at the Baluchistan Institute of Nephro-Urology (BINUQ), Quetta. The BINUQ is the only Nephro-Urology specialized hospital in the city and bears the complete burden of patients having Nephro-Urology issues. A universal sampling approach was adopted and all patients presenting with complaints of UTIs between January 2022 and December 2022 were included in the study to investigate the bacterial profile and antibiotics susceptibility pattern. A total of 1554 samples were acquired based on a subjective symptom-based questionnaire (data not shown), of which 1048 were later verified microbiologically as positive UTI cases.

2.2 Exclusion criteria

Patients <18 years of age, having polymicrobial infections, pregnant females, and those on antibiotic therapy were excluded from the study.

2.3 Sample collection and bacterial identification

The patients were trained by the paramedics on how to collect clean-catch midstream urine. The urine was collected in a sterile screw-capped universal container that was provided by the hospital. Following the collection, the samples were cultured in cystine lactose electrolyte deficient agar. The isolated bacteria were identified by the standard conventional procedure in urine cultures (surface streak procedure; Oxoid Ltd. Bashingstore Hampaire, UK) at 37°C for 18–24 hours. The isolated bacteria were identified using catalase, bile esculin test, and oxidase test. All isolated bacteria were preserved at room temperature (20 to 25°C) for both summers and winter.

2.4 Antibiotic susceptibility method

We employed the disk diffusion method for antibiotic susceptibility 18). The positive samples were subjected to the most used antibiotics (in-vitro) for UTI treatment and are discussed in the result section.

2.5 Ethical approval, consent of participation, and publication

This study was conducted under the Declaration of Helsinki and was approved by the Institutional Review Board of the Faculty of Pharmacy & Health Sciences, University of Balochistan, Quetta (FoP&HS/IRB/43/22). Permission to conduct the study was also obtained from the Chief Executive Officer of BINUQ. Written consent for participation and publication was also taken from the patients.

2.6 Statistical analysis

The Statistical Package for Social Sciences v. 26 was used for data analysis. Based on the objectives of the study, descriptive statistics were used for data elaboration.

3. Results

3.1 Demographic profile

The demographics data are presented in **Table 1**. Out of 1554 suspected samples, bacterial growth was observed in 1048 samples. The remaining 506 samples yielded no growth after 72 hours of culture. Most of the patients were in the age group of 28-37 years (440, 72.0) followed by > 47 (25.5%). Interestingly, as UTI is generally common among women, cohort of the current study was dominated by males (642, 61.3%) when compared with their counterparts.

Sample characteristics and isolated bacteria

The sample characteristics and isolated bacteria are elaborated in Table 2. Three bacteria were isolated during the culture. Seventy-three percent of the isolated bacteria were Escherichia coli (E. coli) followed by Pseudomonas aeruginosa (202, 19.3%) as shown in **Table 2**.

3.2 Types of antibiotics used for antibiotic susceptibility testing

The types of antibiotics used for antibiotic susceptibility testing are presented in Table 3. Twenty different antibiotics were used for the sensitivity testing. The antibiotics with their classes and ATC codes are mentioned in **Table 3**.

Table 1. Demographic characteristics of the study respondents

Characteristics	Frequency (N)	Percentage (%)
Age (38.38±15.25)		
18-27	244	23.3
28-37	440	42.0
38-47	97	9.3
> 47	267	25.5
Gender		
Male	642	61.3
Female	406	38.7

Table 2. Sample characteristics and isolated bacteria

Characteristics	Frequency (N)	Percentage (%)
Specimen received		
Urine	1048	100.0
Isolated organism		
Escherichia coli	768	73.3
Klebsiella species	78	7.4
Pseudomonas aeruginosa	202	19.3

Table 3. Types of antibiotics used for antibiotic susceptibility testing

Name	Group	ATC Code
Cell wall synthesis inhibitors		
Amoxicillin	Penicillin	J01CA04
Ampicillin	Penicillin	J01CA01
Co-amoxiclav	Penicillin	J01CR02
Amoxicillin	Penicillin	J01CA04
Cefotaxime	Cephalosporin	J01DD01
Ceftazidime	Cephalosporin	J01DD02
Cefixime	Cephalosporin	J01DD08
Cefepime	Cephalosporin	J01DE01
Cefoperazone	Cephalosporin	J01DD12
Ceftriaxone	Cephalosporin	J01DD04
Protein synthesis inhibitors (30-S)		
Amikacin	Aminoglycoside	J01GB06
Gentamycin	Aminoglycoside	J01GB03
DNA gyrase inhibitor		
Ciprofloxacin	Fluroquinolones	J01MA02
Norfloxacin	Fluroquinolones	J01MA06
Ofloxacin	Fluroquinolones	J01MA01
Nalidixic acid	Quinolone	J01MB02
Others		
Fosfomycin	Phosphonic	J01XX01
Meropenem	Carbapenem	J01DH02
Tazobactam	β-lactamase inhibitor	J01CR05
Vancomycin	Glycopeptide	A07AA09

3.3 Antibiotics susceptibility pattern

The susceptibility frequency of the antibiotics is presented in **Table 4 (a-d)**. As mentioned, Escherichia coli showed high sensitivity against Meropenem (94.2%) followed by Amikacin (94.1%), Tazobactam (93.5%), Fosfomycin (91.6%) and Vancomycin (90.5%). Similarly, Klebsiella species were sensitive to Fosfomycin (91.6%) followed by Meropenem (91.4%), Tazobactam (90.9%), Amikacin (90.5%) and Vancomycin (84.3%). Pseudomonas aeruginosa showed the highest sensitivity against Meropenem (92.6%) followed by Vancomycin (92.1%), Tazobactam (88.1%), Amikacin (84.4) and Fosfomycin 78.9%.

Escherichia coli had highest resistance against Cefixime (100%) followed by Norfloxacin (94%), Nalidixic acid (93.7%), Ampicillin (85.3%), and Ciprofloxacin (75.1%). Similarly, Klebsiella species were resistant against Cefixime (100%) followed by Nalidixic acid (92.4%), Norfloxacin (89.3%), Ampicillin (88%) and Cefotaxime (60%). Pseudomonas aeruginosa

was also resistant to Cefixime (100%) followed by Nalidixic acid (95.4%), Norfloxacin (87.3%), Ampicillin (77.8%) and Gentamycin (64.2%).

Table 4 (a). Antibiotics susceptibility pattern (Sensitivity)

Isolated bacteria *	Sensitivity pattern								
	AMK**	AMX**	AMP**	AMC**	CTX**	CRO**	CPFX**	CAZ**	FOF**
	N (%)								
E. Coli	400 (94.1)	86 (49.1)	23 (14.7)	193 (61.4)	106 (29.6)	140 (26.1)	126 (22.3)	121 (49.1)	560 (91.6)
K. Spc	202 (90.5)	-	15 (12.0)	85 (47.2)	45 (26.6)	100 (40.4)	110 (40.7)	90 (18.6)	110 (91.6)
P. Arg	136 (84.4)	-	25 (22.1)	115 (61.4)	55 (38.1)	70 (49.3)	60 (48.0)	132 (47.6)	90 (79.0)

E. Coli = Escherichia coli; K. Spc = Klebsiella species; P. Arg = Pseudomonas aeruginosa

***AMK = Amikacin; AMX = Amoxicillin; AMP = Ampicillin; AMC = Amoxicillin/clavulanic acid; CTX = Cefotaxime; CRO = Ceftriaxone; CPFX = Ciprofloxacin; CAZ = ceftazidime; FOF = Fosfomycin*

– means that this bacterium was not tested against that antibiotic

Table 4 (b). Antibiotics susceptibility pattern (Sensitivity)

Isolated bacteria*	Sensitivity pattern									
	GEN**	MEM**	NOR**	NAL**	TZP**	FEP**	CPFX*	OFX**	CFP**	VAN**
	N (%)									
E. Coli	230 (70.7)	411 (94.2)	10 (6.0)	8 (6.2)	463 (93.5)	0	11 (52.3)	8 (44.4)	8 (44.4)	412 (90.5)
K. Spc	141 (71.5)	214 (91.4)	6 (10.7)	7 (7.6)	211 (90.9)	0	10 (47.6)	7 (36.8)	6 (49.1)	242 (87.3)
P. Arg	124 (64.2)	228 (92.6)	7 (12.2)	3 (4.6)	89 (88.1)	0	8 (30.7)	7 (30.4)	10 (71.4)	140 (92.1)

E. Coli = Escherichia coli; K. Spc = Klebsiella species; P. Arg = Pseudomonas aeruginosa

***GEN = Gentamycin; MEM = Meropenem; NOR = Norfloxacin; NAL = Nalidixic acid; TZP = Tazobactam; FEP = Cefixime; CPFX = Cefepime; OFX = Ofloxacin; CFP = Cefoperazone, VAN = Vancomycin*

Table 4 (c). Antibiotics susceptibility pattern (Resistance)

Isolated bacteria *	Sensitivity pattern								
	AMK**	AMX**	AMP**	AMC**	CTX**	CRO**	CPFX**	CAZ**	FOF**
	N (%)								
E. Coli	25 (5.9)	89 (50.9)	133 (85.3)	105 (33.4)	225 (63.0)	358 (66.9)	423 (75.1)	125 (50.9)	51 (8.4)
K. Spc	20 (8.9)	-	110 (88.0)	87 (48.3)	102 (60.3)	121 (48.9)	155 (57.4)	95 (51.4)	10 (8.4)
P. Arg	23 (14.4)	-	88 (77.8)	61 (32.6)	74 (513)	51 (36.0)	65 (52.0)	145 (52.4)	24 (21.1)

E. Coli = Escherichia coli; K. Spc = Klebsiella species; P. Arg = Pseudomonas aeruginosa

***AMK = Amikacin; AMX = Amoxicillin; AMP = Ampicillin; AMC = Amoxicillin/clavulanic acid; CTX = Cefotaxime; CRO = Ceftriaxone; CPFX = Ciprofloxacin; CAZ = ceftazidime; FOF = Fosfomycin*

– means that this bacterium was not assessed against that antibiotic

Table 4 (d). Antibiotics susceptibility pattern (Resistance)

Isolated bacteria*	Sensitivity pattern									
	GEN**	MEM**	NOR**	NAL**	TZP**	FEP**	CPFX*	OFX**	CFP**	VAN**
	N (%)									
E. Coli	95 (25.3)	25 (5.8)	154 (94.0)	121 (93.7)	32 (6.5)	14 (100)	10 (47.6)	10 (55.6)	10 (55.6)	43 (9.5)
K. Spc	56 (28.5)	20 (8.6)	50 (89.3)	85 (92.4)	20 (9.1)	10 (100)	11 (52.3)	12 (63.2)	6 (46.1)	35 (12.7)
P. Arg	69 (35.8)	18 (7.4)	50 (87.3)	61 (95.4)	12 (11.9)	15 (100)	18 (69.3)	16 (69.6)	7 (53.9)	12 (7.9)

E. Coli = Escherichia coli; K. Spc = Klebsiella species; P. Arg = Pseudomonas aeruginosa

***GEN = Gentamycin; MEM = Meropenem; NOR = Norfloxacin; NAL = Nalidixic acid; TZP = Tazobactam; FEP = Cefixime; CPFX = Cefepime; OFX = Ofloxacin; CFP = Cefoperazone, VAN = Vancomycin*

4. Discussion

The current study aimed to identify the antibiotic susceptibility pattern against UTIs among patients attending a specialized Nephro-Urology hospital. The three isolated bacteria were Escherichia coli (E. coli), Klebsiella species (K. spc) and Pseudomonas aeruginosa (P. Arg) with E. Coli being the dominating bacteria in 75% of the cases. A few isolates of Proteus (n=2) and Staphylococcus aureus (n=1) were also identified but their frequency was not germane when compared with the earlier three (<1%). The isolated bacteria are not surprising and are known to cause UTIs around the globe (3). However, an encouraging finding of the current study is that no other strain of bacteria was identified thus encouraging our focus on the traditional causative agents of UTIs.

An interesting finding of the study revealed that UTIs were more reported in men than women. It is evident from the literature that women are more liable to get UTIs (in some cases >30 times) when compared with men. Likewise, women with UTIs are again suspectable of another episode of UTIs within the coming 6 months. The answer to this increased frequency is also straightforward. As women's urethra is smaller, the movement of bacteria into the bladder is rapid compared to men's (19).

On the contrary, the only two justifications for UTIs being more prevalent in men can be linked to our study results. Men with prostate problems especially benign prostatic hyperplasia often block the urine flow. This causes a build of urine in the bladder and there are chances that UTIs will develop. Also, the chances of developing UTIs with a sexually transmitted disease are frequent among men compared to women. .

in their review article concluded that cefixime is an effective choice against UTIs with a low side effect profile. The authors mentioned that the drug can be safely utilized in non-complicated UTIs especially in children as a monotherapy. The authors also highlighted that the drug can also give excellent results in UTIs as a switch therapy and can replace an intravenous antibiotic once the condition of the patient has improved(20). Similarly, Chaudhary and colleagues reported cefixime as a safe and tolerable drug in patients with UTIs (21). Al-Huseini et al in their study among pregnant women concluded cefixime as safe drug to be used against UTIs (22), however the current study did not included pragnent women. Several reasons can be attributed to these conflicting results. The Pakistan Antimicrobial Resistance Surveillance System Surveillance Report published in 2019 highlighted a dramatic increase in cefixime-resistance whereby 94% resistance was observed in 2018 comparing it with 18% in 2017. The report correlated the heavy use of cefixime especially for respiratory tract infections without going through a culture sensitivity assessment (23). Based on our observations, the past decade has seen an increase prescribing of cefixime especially in drug-resistant typhoid, lower respiratory tract infections, and UTIs. This irrational prescribing has developed abrupt resistance against cefixime and hence makes the drug ineffective against UTIs, especially uncomplicated UTIs.

All the three isolated bacteria had resistance against Norfloxacin and nalidixic acid.The results of the current study are comparable to the data what is reported by Iqbal et al in their systematic review (24). The probable reason for these corresponding results is associated with the repeated use of norfloxacin and nalidixic acid in Pakistan. Both antibiotics were misused for a prolonged period and that has led to increased resistance. Moreover, the free availability of antibiotics without prescription at community pharmacies is also a significant factor in developing drug resistance. Community pharmacies in Pakistan and especially in the Baluchistan province are following a business-based model and are operated without a qualified pharmacist. Such pharmacies dispense and sell anything, from antibiotics to antidepressants and little effort is needed by the buyers to purchase specialized medicines. Parallel, there is insufficient control over medicine sales and there is poor administrative surveillance of the law enforcers. Although the Drug Regulatory of Pakistan has devised controlled strategies, such strategies are least observed at the community pharmacy levels. The concept of quality use of antibiotics is lacking and has resulted in irrational antibiotic use.

E. coli reported high sensitivity against Meropenem followed by Amikacin and Tazobactam. Comparative results are observed from other studies of the same nature(25-27). The explanation for this is simple. Meropenem, Amikacin and Tazobactam are expensive and available as parenteral form and the people in Baluchistan prefer economical oral antibiotic. The infrequent use is a reason for the increased sensitivity of meropenem and tazobactam against pathogens including uropathogens.

The most convincing finding of the study was the identification of sensitivity of the isolated bacteria against Fosfomycin. Mixed results are extracted from literature where Mughal et al reported 79% sensitivity of Fosfomycin against E. Coli (28), In terms of resistance, all Gohar and colleagues reported high sensitivity (>90%) of Fosfomycin against E. Coli (29). Acceptable sensitivity of Fosfomycin was again reported by Bullens et al where only one isolate was resistant to Fosfomycin (30). Another study from the Balochistan province of Pakistan also reported Fosfomycin as an active antibiotic sensitive against E. Coli uropathogen (14). Within this context, Fosfomycin an old-new antibiotic promises effective treatment as a replacement for resistant antibiotics. Our suggestion is supported by the results of Hassan et al whereby the authors concluded that in the case of simple UTIs, a single dose of Fosfomycin was as effective and tolerable as a five-day course of ciprofloxacin (31).

5. Conclusion

The susceptibility pattern of our study revealed Meropenem, Amikacin, Tazobactam, Fosfomycin,and Vancomycin as effective drugs against UTIs. In contrast, Cefixime, Norfloxacin, Nalidixic acid, Ampicillin, and Ciprofloxacin showed reported high resistance against the isolated bacteria. The high resistance to the commonly prescribed drugs for in UTIs, this leaves clinicians with limited options for drugs used to treat for the treatment of UTIs. Therefore, we conclude that based on the sensitivity results of the current study, Fosfomycin can be used as an alternate therapy for UTIs where resistance to other drugs is apparent. Moreover, being economically affordable and with a lower side effect profile, Fosfomycin will help in combating AR in UTIs

and will place an affordable burden on the healthcare system of Pakistan.

6. Recommendations

Routine culture sensitivity testing must be mandated mandatory before initiation of drug therapy. The use of Fosfomycin needs to be incorporated into the local guidelines and drug prescription should sternly adhere to the guidelines proposed for the treatment of UTIs. We also recommend continuous medical education for the prescribers so that they can prescribe based on updated and evidence-based literature.Additionally, healthcare professionals must be provided with evidence-based information on the updated and most effective treatment of UTIs.

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الملامح البكتيرية ونمط الحساسية للمضادات الحيوية لمسببات الأمراض البولية التي تسبب التهابات المسالك البولية في مدينة كويتا ، باكستان

الخلاصة:

المقدمة: الاستخدام غير العقلاني للمضادات الحيوية ومقاومة المضادات الحيوية ضد مسببات الأمراض البولية يعد كارثة عالمية .ونتيجة لذلك، فمن الضروري تحديد نمط الحساسية للمضادات الحيوية من مسببات الأمراض البولية أثناء إدارة التهابات المسالك البولية .كما أن التعرف على نمط الحساسية لمسببات الأمراض البولية أمر ضروري للعلاج بالمضادات الحيوية التجريبية .تهدف الدراسة الحالية إلى تحديد المظهر الجرثومي ونمط الحساسية للمضادات الحيوية المعزولة من مرضى التهاب المسالك البولية في الجزء الشمالي من بلوشستان في باكستان .**الطرق:** أجريت الدراسة في الفترة ما بين يناير 2022 وديسمبر 2022. وتم جمع عينات البول النظيفة في منتصف مجرى النهر .تم إجراء إجراءات العزلة والحساسية للمضادات الحيوية من خلال المبادئ التوجيهية القياسية للتقنيات الميكروبيولوجية .تم جمع ألف وثمانية وأربعون عينة وتم إنتاج مزرعة بكتيرية إيجابية .**النتائج:** أربعمائة وأربعون مريضا (72٪) تتراوح أعمارهم بين 28 -37 سنة .ويساهم الذكور بنسبة (61.3%) من جميع العينات .تم العثور على الثقافات الميكروبيولوجية في 1048 مريضا مع كون الإشريكية القولونية هي العامل الممرض السائد (1018، 97.1%)، تليها الزائفة الزنجارية 202 (19.3%) اكتشف نمط الحساسية في دراستنا أن السيفيكسيم والنورفلوكساسين وحمض الناليديكسيك كانت المضادات الحيوية ذات أعلى معدلات مقاومة في مسببات الأمراض البولية البكتيرية، بنسبة 100٪، 94.0٪ و 93.7٪، على التوالي . كانتميروبينيموامیكاسینوتازوباكتاموفوسفومايسينوفانكومايسينمن المضادات الحيوية التي تجاوزت معدلات حساسيتها 90٪ .**الاستنتاج:** أظهرت العزلات البكتيرية التي تم تحديدها في الدراسة الحالية مقاومة عالية للسيفيكسيم والنورفلوكساسين وحمض الناليديكسيك (وخاصة الإشريكية القولونية) .وهذا يترك الأطباء أمام خيارات محدودة جدًا من الأدوية لعلاج عدوى المسالك البولية .إن زيادة المقاومة للمضادات الحيوية الموصوفة بشكل شائع في عدوى المسالك البولية تستدعي مراقبة صارمة ومراقبة استخدام المضادات الحيوية في المجتمع.

الكلمات المفتاحية: المضادات الحيوية، قابلية، التهاب المجاري البولية، مدينة كويتا، باكستان، الملف البكتيري

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