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# Prevalence and Antibiograms of Uropathogens from the Suspected Cases of Urinary Tract Infections in Bharatpur Hospital, Nepal

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## ABSTRACT

**Background:** This study was conducted to determine the prevalence and antibiotic susceptibility pattern of the uropathogens among the patients attending Bharatpur Hospital. **Methods:** A laboratory-based cross-sectional study was carried out among the patients attending Bharatpur Hospital from December 2017 to February 2018. Aseptically collected clean catch mid-stream urine samples from 200 clinically suspected patients were cultured and processed for the identification of the uropathogens in the laboratory using standard microbiological procedures. Antibiotic susceptibility test was performed for all the isolates against commonly used antibiotics using the Kirby-Bauer disc diffusion method according to Clinical and Laboratory Standards Institute guidelines 2017. **Results:** Out of 200 samples collected, 59(29.5%) of the samples showed the presence of pathogens causing urinary tract infection (UTI). Among them, 43(72.9%) were Gram-negative and 16(27.1%) were Gram-positive bacteria. UTI was found to be the most prevalent in females compared to the males and in the age group of 21-30 years. *E. coli* (72.0%) and *Staphylococcus epidermidis* (50.0%) were the most predominant Gram-negative and Gram-positive isolates respectively. The isolates were resistant to cefpodoxime (54.2%) and least resistant to gentamicin (10.2%). Twenty (33.9%) isolates were found to be multi-drug resistant (MDR). **Conclusion:** Higher frequency of antibiotic resistance among UTI patients alerts for continuous surveillance to assure effective control of this infection. Awareness of good hygienic practice especially in females and prudent use of antibiotics in case of infection can be suggested.

**Keywords:** antibiotic susceptibility test; *E. coli*; MDR; urine.

## INTRODUCTION

Urinary tract infections (UTIs) are some of the most common bacterial infections affecting 150 million people each year worldwide and are increasing every day.[1] UTIs are the significant cause of morbidity in infant boys/girls, older men, and female of all age group.[2] UTI is the commonest disease among Nepalese population. According to the annual report published by the Department of Health service (2059/2060) morbidity of UTI in Nepal is 1,25,0584.[3] Geographical distributions of UTI patients in Nepal in Eastern, Central, Western, Mid-western and Far-western region of Nepal were 72,426, 85,760, 77,729, 44,382 and 36,414 respectively in 2015/2016.[4]

UTI is also an important cause of bacteremia but with early diagnosis and management of UTI, the incidence of morbidity and life-threatening bacteremia can be reduced.[5] The commonest cause of urinary tract infection is *E. coli.* Other possible pathogens are *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp., *Chlamydia* spp., etc. In the past few years, an increasing trend in the antibiotic-resistant imparted by *E. coli* and *K. pneumoniae* isolates have been observed which is a frightening scenario.[6] UTI is more common in females as compared to males, especially females of the reproductive age group from 15-50 years.[7] This is due to anatomical predisposition, the close approximation of urethra and vagina and sexually active life during this year.[8]

Easy availability and haphazard use of antibiotics such as ampicillin, norfloxacin, and nitrofurantoin are one of the major contributors to drug-resistant in India.[9] Quinolones especially fluoroquinolones are broad-spectrum antimicrobial agents used widely to treat UTI. Among the fluoroquinolones, ciprofloxacin is the most used antibiotic against pathogens commonly encountered in UTIs. It reaches the high concentration in the prostatic tissue and in the seminal fluid; therefore, it is considered to be the first therapeutic choice in males with UTI.[10] In Nepal, the prevalence of UTI pathogens and their antimicrobial resistant rates vary considerably from regions to regions. Therefore, to ensure the appropriate therapy current

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knowledge of organism that causes UTI and their susceptibility pattern is obligatory.[11] Nepal, a developing country, has about 61.4% illiterate people who do not have any concept of good hygiene and so are always vulnerable to infection by the various microorganisms. The annual report of the fiscal year (2055/2056) published by the Department of health services showed that 0.46% of total outdoor patients were UTI victims.[12]

UTI is the most common bacterial infection causing illness in females mostly in developing countries like Nepal due to illiteracy, unhygienic condition and lack of proper toilet facilities.[13] The common source of *E. coli* infection in women is the fecal flora. Internal colonization precedes the development of urinary tract infection in females. In males, the organism frequently originates from sub pepucial sac.[14] UTI is challenging not only because of frequent and high infection rate that occurs each year but also because the diagnosis of UTI is not always straightforward and some UTIs are asymptomatic and difficult to diagnose. The present study was carried out to study the prevalence of UTI among the patients visiting Bharatpur Hospital as well as to identify the most common bacteria causing UTI along with their resistance patterns to commonly used antimicrobials.

## METHODS

### Study site and population

A cross-sectional study was carried out at the Microbiology laboratory of Bharatpur Hospital from December 2017 to February 2018 among the UTI suspected patients attending the Hospital. Data were collected using a semi-structured questionnaire. Systematic random sampling was done to select the required number of samples. Chi-square test ($\chi^2$) was used to determine significant associations between various attributes including age and gender of the patients with the prevalence rate of UTI.

### Sample Size, inclusion criteria and exclusion criteria

A total of 200 mid-stream urine samples were collected from all suspected patients. Samples showing mixed growth and non-significant growth on the culture were rejected and only the samples showing significant growth were included in the study. Urine culture showing greater than 100,000 colony-forming units (CFU) of bacteria per ml of urine was regarded as "significant bacteriuria".[15] Samples with improper labeling were also rejected.

### Collection of samples

The mid-stream urine (10–20ml) samples were collected in the sterile dry, wide-necked, leak-proof container. All the samples were processed within 1 hour.[16]

### Processing of the samples

A loopful of the sample was directly inoculated into CLED (Cysteine Lactose Electrolyte Deficient) Agar and incubated at 37°C for 24 h. No MRSA screening was performed. After Gram's staining, the isolates colonies were subcultured in Tryptic soya agar (TSA), biochemical tests such as IMViC, TSI (Triple Sugar Iron), motility, oxidative-fermentative, nitrate reduction, catalase, oxidase, urease, coagulase, DNase and Dryspot Staphytect Plus for *Staphylococcus aureus* were performed.[17]

### Antibacterial sensitivity tests

The antibacterial susceptibility testing of isolates was done by modified Kirby Bauer disc diffusion method using commercial discs from Himedia.[18] An isolated colony of an organism was suspended in the 0.85% normal saline water. The turbidity was matched with 0.5 McFarland turbidity standards. A sterile cotton swab was taken and introduced into the tube containing the suspension of the organism and swabbed uniformly on the surface of Mueller Hinton agar medium. The plate was allowed to dry and antibiotic discs were placed on the agar surface. Gentamicin (30 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), cotrimoxazole (25 µg) and cefpodoxime (10 µg) discs were used to identify antibacterial susceptibility pattern of the isolates, and novobiocin (30 µg) was used only for *Staphylococcus* spp. Plates were incubated for 18 to 24 h at 37 ºC. Zone of inhibition was measured in millimeters (mm). Resistance shown to at least three or more antibiotics of different structural classes was considered MDR as described elsewhere.[19,20]

### Quality control for test

For quality control, media, antibiotics, and reagents were prepared, stored and utilized as recommended by the manufacturing company. Antibiotic discs were stored at refrigerator temperature (4 ºC). The accuracy of the overall testing was monitored by using *S. aureus* ATCC 25923 as reference strain and control strains of *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603. For each batch of the test, a positive and negative known culture was used for the color reaction, biochemical test, and antibiotic sensitivity test.

### Data management and analysis

All raw data obtained were tabulated and presented in defined tables to explore the major findings. SPSS 20 version software was used to calculate p-values of the obtained result. P-values less than 0.05 were considered to have a significant association.

## RESULTS

Out of 200 mid-stream urine samples, 59 (29.5%) samples showed growth whereas 141(70.5%)

samples didn't show growth. All the positive samples showed the growth of a single organism (Figure 1).

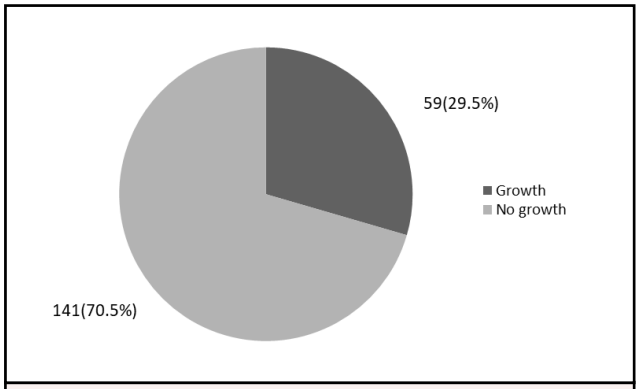


**Figure 1. Growth pattern of urine culture.**

Among 59 bacterial isolates, 43 (72.9%) were Gram -negative and 16 (27.1%) were Gram-positive. Altogether five species of Gram-positive bacteria were isolated during the study. The most prevalent bacteria obtained were *S. epidermidis* 8(47.0%) and *Streptococcus* group D 1 (5.9%) was the least frequent bacteria. Similarly, five species of Gram-negative bacteria were isolated during the study. The most prevalent bacteria obtained was *E. coli* 31 (72.0%) and *Aeromonas hydrophila* 1 (2.3%) was the least frequent bacteria (Table 1).

**Table 1. Distribution of bacteria from urine.**

| Bacterial distribution | No. | % |
|---|---|---|
| **Gram positive bacteria** | **16** | **27.1** |
| *Staphylococcus epidermidis* | 8 | 47.0 |
| *Staphylococcus saprophyticus* | 4 | 23.5 |
| *Staphylococcus aureus* | 1 | 5.9 |
| *Micrococcus* spp. | 2 | 11.8 |
| *Streptococcus* Group D | 1 | 5.9 |
| **Gram negative bacteria** | **43** | **72.9** |
| *E. coli* | 31 | 72.0 |
| *Klebsiella pneumoniae* | 7 | 16.3 |
| *Pseudomonas aeroginosa* | 2 | 4.6 |
| *Enterbacter aeroginosa* | *2* | 4.6 |
| *Aeromonas hydrophila* | 1 | 2.3 |

Out of 200 samples examined in this study, 123 samples were collected from females among which 43 (72.9%) were found to be growth positive and 77 samples were collected from male among which 16 (27.1%) were found to be growth positive. There was a significant association between the prevalence of UTI with the gender of the patient ($p<0.05$) (Table 2).

Age-wise distribution of patients indicates that the highest number of bacteria 17 (28.8%) was isolated in the age group 21-30 years in both the genders. It was then followed by the age group 31-40 years in which 14 (23.7%) bacteria were isolated. Similarly, 10 (16.9%) samples in the age group (0-10) were culture positive. No any bacteria were isolated in the age above 80. There was a significant association between the prevalence of UTI with age of the patients ($p- <0.05$) (Table 3).

**Table 2. Sex-wise distribution of patients.**

| Sex | Total samples | Positive Sample | Percentage (%) | P-value |
|---|---|---|---|---|
| **Male** | 77 | 16 | 27.1 | 0.03* |
| **Female** | 123 | 43 | 72.9 | |
| **Total** | 200 | 59 | 29.5 | |

**Table 3. Age-wise distribution of patients.**

| Age Group (years) | Total samples | Positive sample | Prevalence (%) | P-value |
|---|---|---|---|---|
| 0-10 | 31 | 10 | 16.9 | 0.04* |
| 11-20 | 26 | 9 | 15.3 | |
| 21-30 | 42 | 17 | 28.8 | |
| 31-40 | 49 | 14 | 23.7 | |
| 41-50 | 12 | 1 | 1.7 | |
| 51-60 | 14 | 4 | 6.8 | |
| 61-70 | 11 | 2 | 3.4 | |
| 71-80 | 10 | 2 | 3.4 | |
| Above 80 | 5 | - | - | |
| Total | 200 | 59 | 29.5 | |

The majority of *E. coli* (83.0%) and *K. pneumoniae* (20.3%) were found to be resistant against all the used antibiotics. However, *S. aureus* and *Micrococcus* spp. showed no resistance against all the tested anti-microbials. Isolates were highly resistant to cefpodoxime (54.2%) and least resistant to gentamicin (10.2%) (Table 4). Out of the 59 isolates, 20 (33.9%) of isolates were found to be MDR. All (100%) *Streptococcus* Group D, *P. aeroginosa, E. aerogenes* isolates were found to be MDR. Similarly, 50.0% of *S. saprophyticus* and *K. pneumoniae* were detected MDR. *E. coli* (25.8%) and *S. epidermidis* (25.0%) were found to be MDR (Table 5).

**Table 4. Antimicrobial susceptibility patterns of the isolates.**

| Isolates | Nitrofurantoin | | Gentamicin | | Ciprofloxacin | | Cotrimoxazole | | Cefpodoxime | | Total Isolates | Resistant |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | % | R | % | R | % | R | % | R | % | | |
| *Staphylococcus epidermidis* | 1 | 12.5 | - | - | 1 | 12.5 | 4 | 50.0 | 3 | 37.5 | 9 | 15.2 |
| *Staphylococcus saprophyti-cus* | 1 | 25.0 | 1 | 25.0 | 1 | 25.0 | 2 | 50.0 | 3 | 75.0 | 8 | 13.5 |
| *Staphylococcus aureus* | - | - | - | - | - | - | - | - | - | - | - | 0.0 |
| *Micrococcus* spp. | - | - | - | - | - | - | - | - | - | - | - | 0.0 |
| *Streptococcus* Group D | 1 | 100.0 | - | - | 1 | 100.0 | 1 | 100.0 | 1 | 100.0 | 4 | 13.5 |
| *E. coli* | 3 | 9.7 | 3 | 9.7 | 11 | 35.0 | 14 | 45.0 | 18 | 58.0 | 49 | 83.0 |
| *Klebsiella pneumoniae* | 2 | 83.3 | 2 | 33.3 | 2 | 33.30 | 3 | 50.0 | 3 | 50.0 | 12 | 20.3 |
| *Pseudomonas aeroginosa* | 1 | 50.0 | - | - | 2 | 100.0 | 1 | 50.0 | 2 | 100.0 | 6 | 10.2 |
| *Enterbacter aerogenes* | - | - | - | - | 2 | 100.0 | 2 | 100.0 | 2 | 100.0 | 6 | 10.2 |
| *Aeromonas hydrophila* | - | - | - | - | 1 | 100.0 | - | - | - | - | 1 | 1.7 |
| Total | 9 | 15.2 | 6 | 10.2 | 21 | 35.6 | 27 | 45.8 | 32 | 54.2 | - | - |

**Table 5. Multi-Drug Resistant Patterns of the isolates.**

| Isolates | No. of Isolates | MDR | % |
|---|---|---|---|
| *S. epidermidis* | 8 | 2 | 25.0 |
| *S. saprophyticus* | 4 | 2 | 50.0 |
| *S. aureus* | 1 | - | - |
| *Micrococcus* spp. | 2 | - | - |
| *Streptococcus* Group D | 1 | 1 | 100.0 |
| *E. coli* | 31 | 8 | 25.8 |
| *K. pneumoniae* | 5 | 3 | 50.0 |
| *P. aeroginosa* | 2 | 2 | 100.0 |
| *E. aerogenes* | 2 | 2 | 100.0 |
| *A. hydrophila* | 1 | - | - |
| Total | 59 | 20 | 33.9 |

## DISCUSSION

In the present study, out of 200 urine samples, 59 (29.5%) samples showed the growth of significant bacteria, which is almost similar to the result obtained in a study from Kanti Children Hospital, Nepal showing 28.0% culture positive results.[21] In contrast, the present study showed a higher bacterial growth than a study conducted by Raghubanshi et al. (2014), at KIST Medical College Teaching Hospital, Nepal accounting 18.5% of positive results[5] and the study conducted by Awasthi et al. (2015) in Seti Zonal Hospital, Dhangadi, Nepal showing 25.5% positive results.[22] Taneja et al. (2010) found 22.2% positive rate in a tertiary care center from north India which is lower than our result.[23] Similarly, the work carried out by Chithra and Meenakshi (2016) reported 34.3% prevalence rate.[24] Such difference in the growth might be due to various reasons such as the difference in geographical distribution, climatic condition, host factor, socioeconomic standard, education program, lack of good hygiene practice, etc.

Among 59 bacterial isolates, 43(72.9%) were Gram -negative and 16(27.1%) were Gram-positive. In this study, Gram-negative bacteria were more prevalent than Gram-positive bacteria. The study by Shrestha et al. (2007) in Nepal reported that among the total 80 bacterial isolates, 75(93.8%) was Gram-negative and only 5(6.3%) were Gram-positive bacteria.[25] Similarly, Karki et al. (2004) in Nepal found 91.1% of isolates were Gram-negative and 8.8% of them were Gram-positive.[26] Similar findings have been reported by Moyu et al. (2010) at Muhimbili National Hospital in Tanzania who showed 61.9% of isolates were Gram-negative and 38.1% were Gram-positive.[27]

In this study, out of 59 isolates, 16 were Gram-positive. *S. epidermidis* 8(50%) was the leading one followed by *S. saprophyticus* 4(25%), *S. aureus* 1 (6.25%) and *Streptococcus* group D 1(6.25%). A similar study by Moyo et al. (2010) in Tanzania reported the prevalence of coagulase-negative *Staphylococcus* 7(16.7%) and *S. aureus* 6(14.3%) in UTI patients.[27] Another study done by Awasthi et al. (2015) reported a prevalence of 4.0% *S. aureus* in the UTI patients at Seti-zonal Hospital Dhangadi, Nepal.[22] In their study, only one type of Gram-positive bacteria-*S. aureus* was isolated. However, our study revealed four other Gram-positive bacteria besides *S. aureus.*

Among Gram-negative isolates, *E. coli* 31(72.0%) was found to be the most predominant organism followed by *K. pneumoniae* 7(16.3%), *P. aeruginosa* 2(4.6%), *E. aerogenes* 2(4.6%) and *A.hydrophila* 1(2.3%). In a similar study done by Khanal et al. (2006) in Nepal, out of 41 isolates, 8 different species were isolated among which *E. coli* 65.8% was found to be most predominant followed by *K. pneumoniae* (9.7%).[28] Awasthi et al. (2015) also showed a high prevalence of *E. coli* (53.1%) in the western part of Nepal.[22] Other previous studies also found that the commonest invading agent in UTI is *E.coli*.[29-30] In Dalhatu Araf Specialist Hospital, Nigeria, Kolawole et al. (2009) reported *E. coli* (30.6%) was the most prevalent pathogen causing UTI.[14] But the study done at a single institution in Japan during the 20 year period of the study, *P. aeruginosa* was ranked as the most frequently isolated pathogens among Gram-negative bacteria.[31] The high prevalence of *E. coli* in causing UTI may be due to its higher binding capacity to the glycoconjugate receptor of the uroepithelial cells of the human urinary tract with its unique virulence determinant, the p pilus (Gal-Gal receptor).[32]

In the present study, out of 200 samples, 77 samples were taken from the male patients, among which 16 (27.1%) samples showed the positive result and out of 123 samples taken from the female, 43(72.9%) samples showed the positive result. There was a significant association between the prevalence of UTI with the sex of the patient ($p < 0.05$) in the study. This result showed that UTI is more common in female than the male which is consistent with the research conducted by Shrestha et al (2007) who reported the culture positivity of 29.8% in females and 15.2% in males.[25] In a similar study by Baral et al. (2012) the culture positivity was 33.5% among female patients and 23.7% in male patients.[33] Raghubanshi et al. (2014), however, reported that overall UTI was more prevalent in male children (52.6%) in all age groups except in 5-15 years.[5] This was a Hospital-based study and probable gender bias in seeking medical treatment could be possible reasons for the male being common in UTI. The higher rate of UTI among the females was related to their anatomical and physiological relationship that means shorter and wider urethra. The anatomical relationship of the female urethra

and the vagina makes it liable to trauma during sexual intercourse as well as bacteria been massaged up to urethra into the bladder during pregnancy and childbirth.[34] Similarly, the hormone plays an important determinant of UTI. Lowering the level of estrogen at the time of menopause leads to conversion of the vaginal flora from *Lactobacillus* spp. to *E. coli*, thus increasing the incidence of UTI.[35]

The present study showed that the more susceptible age group for UTI was 21-30 years with prevalence 28.8% followed by the age group 31-40 (23.7%) and unsusceptible age group was more than 80 years ($p < 0.05$). This might be due to reason that reproductive age group has a high prevalence rate of UTI and incidence of symptomatic UTI is high in sexually active young groups.[36] A study done by Thapa et al. (2013) at Tertiary care Hospital of Western Nepal reported that the highest susceptible age group was 20-30 years which had the prevalence of 28.8% and the least was found in age group more than 80 years.[37] Previous studies done at B and B Hospital, Nepal also reported that the more susceptible age group among the male was 20 -30 years and among the female was 30-40 years.[34] But the study performed by Shigemura et al. (2005) at a single institution in Japan during a 20 year period reported that most of the uropathogens were recorded in subjects aged 50 years and above. The high prevalence rate of UTI observed in the aged people may be solely due to the inability of their immune system to fight or resist bacterial infection.[31]

In a study done by Thapa et al. (2013) at Tertiary care Hospital of Western Nepal, it was reported that isolates showed higher percentage of resistance against ampicillin (75.0%), followed by cotrimoxazole (58.0%), ciprofloxacin (25.0%), gentamicin (9.0%), nitrofurantoin (5.0%), amikacin (4.7%).[37] In our study, antibiotics susceptibility tests of isolates revealed the higher percentage of resistant against cefpodoxime (54.2%) followed by cotrimoxazole (45.8%), ciprofloxacin (35.6%), nitrofurantoin (15.2%) and gentamicin (10.2%). A work by Sharma et al. (2013) in Bir Hospital Nepal showed fewer isolates resistant to nitrofurantoin (5.5%) than in our study.[6] However, as compared to our research, they reported isolates showing higher evidence of resistance against cefpodoxime (54.2%) and cotrimoxazole (45.8%). Our study revealed that *E. coli* (31.6%) and *K. pneumoniae* (34.3%) were found to show resistance against the used antibiotics whereas *S. aureus* and *Micrococcus* spp. showed no resistance against all the tested antimicrobials. Antimicrobial resistance is a big problem drawing a great concern throughout the world. Resistance to antibiotics develops due to its frequent misuse.[38-40]

The present study showed the presence of 20 (33.9%) MDR bacteria which is less than the results found in the several studies conducted by Awasthi et al. (2015) in Seti Zonal Hospital, Dhangadi, Nepal (42.9%),[22] and Khanal et al. (2006) in Teaching Hospital, Kathmandu, Nepal (56.1%).[28] In our study, *E. coli* (25.8%), *K. pneumoniae* (42.8%) and *P. aeroginosa* (100.0%) were found to be MDR. However, Awasthi et al. (2015) reported, 48.1% of the *E. coli*, 19.0% of the *K. pneumoniae,* 50.0% of the *P. aeruginosa,* as MDR isolates.[22] Their study showed some *S. aureus* (25.0%) were MDR but no MDR *S. aureus* was detected in our study. A research work conducted by Baral et al. (2012) in Kathmandu Nepal found that 50.0% of the coagulase-negative staphylococci developed MDR,[33] whereas our study reported *S. epidermidis* (25.0%) and *S. saprophyticus* (50.0%) were MDR. They also reported 1(100.0%) MDR *S. aureus.* In contrary to our study, their study did not report MDR *Streptococcus* Group D and *E. aerogenes.* The prevalence of multi-drug resistant organisms is soaring every day and can have terrible penalties. This problematic issue is attributed to two main reasons: growing haphazard use of antibiotics and sales of substandard drugs.[21] The presence of such a tremendous number of MDR bacteria in causing UTI is very serious which cannot be ignored. Therefore, crucial initiatives should be taken from responsible authorities and policymakers to control this problem in time.

## CONCLUSIONS

This study shows the high prevalence of multidrug resistance among bacterial uropathogens. Particularly, the rate of resistance to cefpodoxime, cotrimoxazole, ciprofloxacin was higher and these antibiotics should be limited and wisely used for empirical-based therapy of UTIs. Awareness of good health practice and health education about UTI should be strongly recommended. Antimicrobial stewardship programmes and antibiograms should be developed by healthcare institutions to reduce appropriate antimicrobial use, improve patient outcomes and reduce the adverse consequence of antimicrobial use.

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