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Use of Turmeric Against *Escherichia coli* and *Staphylococcus aureus* Isolated from Urinary Tract Infections: A Laboratory-Based Study

Anjila Pal[1], Bhim S Mahawal[1], Rabin Bom[2]

Author(s) affiliation

[1]*Department of Microbiology, Lumbini Medical College & Teaching Hospital, Palpa, Nepal*

[2]*Department of General Practice and Emergency Medicine, Maharajgunj Medical Campus, Tribhuvan University, Teaching Hospital, Institute of Medicine, Kathmandu*

Corresponding author

Anjila Pal, MSc
anjilapal789@gmail.com

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ABSTRACT

Introduction

Urinary tract infection (UTI) is an important global health problem affecting millions of peoples annually; more common among females. Among the various bacterial etiologies for UTIs, *Escherichia coli* is the commonest one. Turmeric has been used since ancient times as a home remedy for various medical conditions. The aim of our study is to determine the prevalence of *E. coli* and *Staphylococcus aureus* associated UTIs, their antibiogram and antibacterial effects of aqueous extracts and discs of turmeric against these isolates.

Methods

All the urine samples were cultured and the isolates were identified as *E. coli* and *S. aureus* based on standard microbiological tests. Different turmeric extracts were prepared using ethanol, methanol and distilled water as solvents. Turmeric discs were also prepared by soaking the sterile filter paper discs in the different turmeric extracts. The isolated *E. coli* and *S. aureus* were tested for antibacterial activity against turmeric extracts and discs by using agar well diffusion and disc diffusion assays.

Results

Out of 240 urine samples, 90 showed growth with 26.67% (24) and 5.55% (5) isolates of *E. coli* and *S. aureus* respectively. Most of the isolates were isolated from females of the age group 41-60 years. No zone of inhibition was observed for any of the bacterial isolates as well as the standard ATCC strains of *E. coli* and *S. aureus* against all the turmeric extracts. Nitrofurantoin and Gentamicin were most effective antibiotics against *E. coli* and *S. aureus* respectively.

Conclusion

This study concluded that no antibacterial activity was seen for bacteria isolated from suspected cases of UTIs namely; *E. coli* and *S. aureus* against different turmeric extracts and discs.

Keywords
Antibacterial activity, turmeric, UTI

INTRODUCTION

Turmeric (*Curcuma longa*), commonly called 'Besar' in Nepali, is a brownish-yellow colored compound and a native of South-East Asia where it is used as a food spice, preservative and coloring agent.[1,2] Not only this, turmeric is extensively used in Ayurveda, Unani and Siddha medicine as home remedy for various diseases since ancient times.[3]

After the identification of curcumin as the main constituent of turmeric, multiple pharmacological activities of curcumin like anti-microbial, anti-diabetic, anti-inflammatory, anti-carcinogenic, anti-coagulant, anti-fertility and wound healing activities have been reported.[1,3–5]

Microbiologically, urinary tract infection (UTI) is established when pathogenic microorganisms are grown in cultures of properly collected midstream "clean catch" urine sample with a count of $\geq 10^5$ organisms per ml.[6] UTI is caused by microbial invasion of the genitourinary tract. UTI is an extremely common condition that occurs in both the sexes at all ages.[7,8] Despite easy availability of antibiotics, UTI remains the most common bacterial infection occurring from the neonatal to the geriatric age group. Antibiotics are usually prescribed empirically which may develop resistant strains of uropathogens.[9]

Escherichia coli is associated with 90% of community-acquired and upto 50% nosocomial UTIs. Further, it can also cause neonatal meningitis, sepsis and systemic infections.[7,10] *Staphylococcus aureus* is also a major community- and hospital-acquired pathogen that causes a broad spectrum of diseases, ranging from soft-tissue infections to life threatening deep tissue damage, and even more invasive diseases, such as bloodstream infections, endocarditis, pneumonia, septicemia, post-operative wound infections and device-related infections.[11–13]

In microbiology, *E. coli* is one of the most frequently isolated bacteria among the Gram-negative bacteria. Similarly, *S. aureus* is the most common Gram-positive bacteria associated with various bacteriological infections. The aim of the study is to test the efficacy of turmeric as an antibacterial agent against the most frequently isolated bacterial pathogens.

METHODS

A descriptive cross-sectional study was conducted over a period of six months from August 2020 to January 2021 in the clinical laboratory of the Department of Microbiology of Lumbini Medical College & Teaching Hospital (LMCTH). All the urine samples received for culture were included in the study. Non-probability (consecutive) sampling technique was used as a sampling technique. At 95% confidence level, taking 80.9% as the prevalence of *E. coli*,[7] and maximum tolerable error (e) = 0.05 the sample size was calculated as 236.4 by using the formula, $n = Z\alpha^2PQ/e^2$. A total of 240 urine samples were included in the study irrespective of the department, age and gender of the patients. The data were entered in SPSS version 21.0 and Excel 2016 and then analyzed. Specimens that are improperly labelled, kept at room temperature for over 2 hours and giving insignificant growth were excluded to reduce bias in the study result.

Recently harvested turmeric powder was purchased from local villager of Pravas, Palpa. Ten grams of turmeric powder was dissolved in each of the three solvents; distilled water, ethanol and methanol to make 50ml of aqueous, ethanolic and methanolic extracts (20% w/v) respectively in sterile beakers. The mixture was kept undisturbed at room temperature for 24 hours with the beakers covered with aluminum foil to avoid evaporation. Each of the solutions were filtered through sterile Whatman No.1 filter paper. After filtration, the filtrate was evaporated in water bath at 25 ºC until half extract was left in the beaker.[14]

Whatman filter paper No.1 was punched into 6mm diameter discs using punching machine. The discs were kept in petri plates, wrapped with aluminium foil and sterilized in hot air oven at 160 ºC for 2 hours. The filter paper discs were soaked in different turmeric solutions for 24 hours to ensure full saturation of the discs. The discs were aseptically removed from each solution using alcohol flamed forceps, placed in petri plates and dried in hot air oven at 25 ºC. The turmeric discs were aseptically packed in sterile bottles labelled with the name of solvent and stored in the refrigerator for future use for testing antibacterial activity of turmeric. 15 Negative control discs were prepared by soaking sterile filter paper discs in sterilized distilled water using same procedure as above. Controls for ethanol and methanol were not used.

Urine was inoculated on cysteine lactose electrolyte deficient (CLED) agar MacConkey agar and Blood agar by semi-quantitative method to demonstrate significant bacteria i.e. $>10^5$ colony forming unit per milliliter (CFU/ml). The media after inoculation were incubated aerobically at 37˚C for 24-48 hours. The bacterial colonies showing growth of $>10^5$ CFU/ml were considered for further identification. Bacterial isolates were identified based on standard microbiological criteria such as colony characteristics, gram staining, and biochemical properties. *E. coli* was identified by Catalase test, Oxidase test, Indole test, Methyl Red test, Voges Proskauer test, Citrate test, Triple Sugar Iron test and Urease test whereas *S. aureus* was identified by Catalase and Coagulase test. Information regarding

Table 1. Antibacterial activity of different turmeric extracts in different solvents against test bacteria by agar well diffusion and disc diffusion methods

Bacteria (n)	Diameter of inhibition zone (in mm)			Negative control
	Aqueous extract (%)	Ethanolic extract (%)	Methanolic extract (%)	
E. coli (24)	Nil	Nil	Nil	Nil
S. aureus (5)	Nil	Nil	Nil	Nil

patient's age, gender and origin (OPD, emergency or ward) was recorded for all cultures where *E. coli* and *S. aureus* were grown as significant pure or predominant organisms.

The 0.5 McFarland standard turbidity matched colony suspension of the isolated bacteria were swabbed on Mueller Hinton Agar (MHA) plates using a sterile cotton swab. Agar wells were prepared using a sterilized cork borer of 6 mm diameter. Using a micropipette, 20 µl of different turmeric extracts (ethanolic, methanolic and aqueous), obtained after evaporation, were added to different wells labelled with the solvent names on the petri plate. Sterilized distilled water was used as negative control instead of turmeric extract and was added to one of the wells. The plates were kept at room temperature for ten minutes to allow the diffusion of the extract into the agar. The plates were then incubated aerobically at 37 ºC for 24 hours in upright position. The antibacterial activity if present was seen as inhibition zone surrounding the well containing turmeric extracts. The diameter of zone of inhibition was measured and expressed in millimeters. The inhibition zones of <9 mm diameter were considered as having no antibacterial activity or inactive, 9-12 mm as partially active, 13-18 mm as active and >18 mm as very active for both disc and agar cup methods.[14,16] Filter paper disc diffusion method was also performed for all the bacterial isolates on MHA by the standard modified Kirby Bauer disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines. Using ethanol dipped and flamed forceps, the different turmeric discs were placed over MHA plates swabbed with isolates of *E. coli* and *S. aureus* in respective plates. Filter paper disc soaked in sterilized distilled water was used as negative control. The plates were kept at room temperature for 10 minutes to allow diffusion of turmeric extract into the agar. The plates were then incubated aerobically at 37 ºC for 24 hours. Next day, the diameter of zone of inhibition was measured and results recorded. For comparing between the antibacterial activity of turmeric, the isolates were also tested for their antibiotic sensitivity against different antibiotics (Hi Media, Laboratories, Pvt. Limited, Mumbai, India). Antibiotic discs such as ampicillin (10 mcg), cefazolin (30 mcg), cotrimoxazole (25 mcg), gentamicin (30 mcg), nitrofurantoin (300µg), norfloxacin (10 mcg) and ofloxacin (5 mcg) were used for antibiotic susceptibility tests of *E. coli*. And discs that were used for *S. aureus* were ampicillin (10 mcg), cefoxitin (30 mcg), ciprofloxacin (5 mcg), cloxacillin (10 mcg), gentamicin (30 mcg), norfloxacin (10 mcg) and ofloxacin (5 mcg). *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as reference strains for quality control of antibiotic susceptibility test of different antibiotic discs.

RESULTS

Out of 240 urine samples suspected of UTIs, 90 (37.50%) showed growth. A total of 24 *E. coli* and 5 *S. aureus* were isolated from 240 urine samples.

Among the isolates, most were isolated from the females of reproductive age group i.e. 21-60 years (20, 83.33%). All the three extracts of turmeric as well as discs were ineffective against both the test organisms (24 *E. coli* and 5 *S. aureus*) as shown in Table 1.

The antibacterial activity of different turmeric extracts against the standard *E. coli* ATCC 25922

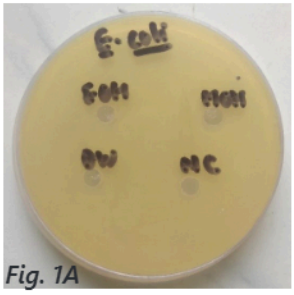

Fig. 1A

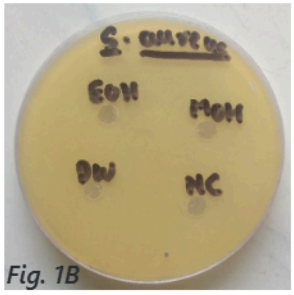

Fig. 1B

Figure 1. A: Agar well diffusion against Escherichia coli B: against Staphylococcus aureus

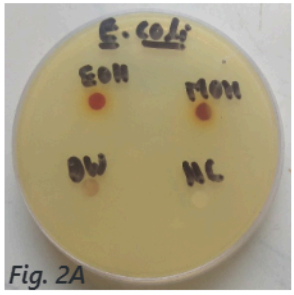

Fig. 2A

Fig. 2B

Figure 2. A: Agar disc diffusion against Escherichia coli B: against Staphylococcus aureus

Table 2: Antibiotic sensitivity pattern of E. coli

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin	7 (29.1%)	0 (0%)	17 (70.8%)
Cefazolin	9 (37.5%)	0 (0%)	15 (62.5%)
Cotrimoxazole	12 (5%)	0 (0%)	12 (50%)
Gentamicin	22 (91.6%)	0 (0%)	2 (8.3%)
Nitrofurantoin	23 (95.8%)	1 (4.1%)	0 (0%)
Norfloxacin	14 (58.3%)	1 (4.1%)	9 (37.5%)
Ofloxacin	14 (58.3%)	0 (0%)	10 (41.6%)

Table 3: Antibiotic sensitivity pattern of S. aureus

Antibiotics	Sensitive (%)	Resistant (%)
Ampicillin	4 (80%)	1 (20%)
Cefoxitin	4 (80%)	1 (20%)
Ciprofloxacin	4 (80%)	1 (20%)
Cloxacillin	4 (80%)	1 (20%)
Gentamicin	5 (100%)	0 (0%)
Norfloxacin	4 (80%)	1 (20%)
Ofloxacin	3 (60%)	2 (40%)

strain and *S. aureus* ATCC 25923 strain was tested as shown in the Figures 1a and 1b respectively. But the extracts did not show any antibacterial activity against the two strains.

The different turmeric discs also did not show any antibacterial activity against the two standard ATCC strains of as shown in the Figures 2a and 2b.

Among the seven different antibiotics tested against all the *E. coli* isolates, nitrofurantoin was found to be the most sensitive antibiotic with a sensitivity of 95.83% as shown in Table 2. This was followed by gentamicin being 91.67% sensitive. In the other hand, most of the isolates were resistant towards ampicillin i.e. 70.83%.

Among the seven different antibiotics, all the isolated *S. aureus* were susceptible to gentamicin (100%) as shown in Table 3. The isolates showed least sensitivity towards ofloxacin (60%) while 80% sensitivity towards remaining five antibiotics.

DISCUSSION

Turmeric is an ancient spice derived from the rhizomes of C. longa. In Nepal, turmeric paste is externally applied to treat paralysis, bruises, pains and injuries in hilly communities[1,17] and added as spices in various foods.[16] It is an essential ingredient for curry powder and is used in manufacture of foods that are consumed in huge quantities such as chips, dairy products, mustards, and cereals.[18]

E. coli was the most predominant etiological agent responsible for causing UTIs (26.67%) in case of Gram negative isolates similar to the findings of Adhikari et al.[19], Manikandan et al.[20] and that in case of Gram positive was *Enterococcus* spp followed by *S. aureus* (5.55%) as in the study done by Shrestha et al.[21] Of the total isolates, 86.2% (25) were isolated from females with 69% (20) being recovered from the reproductive age group indicating that the incidence of UTIs is more common among females, more precisely females of reproductive age group, than males. This finding is analogous with the findings of many studies.[22–24]

The study was done to evaluate the antibacterial activity of different turmeric extracts against common uropathogens causing UTIs. This study showed no antibacterial activity against all the tested *E. coli* and *S. aureus* isolated during the study period. Similar findings were reported from Nepal by Sah et al.[16]; and Maharjan et al.[25] showed slight activity against *S. aureus*.

Some studies have also demonstrated the challenges of using turmeric (curcumin) as a therapeutic option; antibiotic. Nabavi et al.[26] demonstrated poor bioavailability of curcumin and its potential photochemical degradation when exposed to UV light. In a joint report released by the Food and Agriculture Organization (FAO) and World Health Organization (WHO) Expert Committee on Food Additives, recommended that the acceptable daily intake (ADI) of curcumin without any possible adverse effects is 0-1 mg/kg body weight. Further, the maximum daily dose of curcumin can be up to 8 grams when administered orally. But, 75% of curcumin was excreted in faeces and considerable amount in urine when 1 g/kg curcumin was administered orally to rats 5. In a human trial done by Sharma et al.[27] with oral administration of curcumin, the patients presented with side effects like nausea and diarrhea. Curcumin was excreted in feces and urine as well. Due to these side effects, few withdrew consent from the study. Teow et al.[28] demonstrated that curcumin treatment may cause cancers even though there are well documented studies on the anticancer action of curcumin. In a study done by Joshi et al.[29] in humans, allergic skin rashes and intercurrent fever were seen as side effects of turmeric oil treatment thus the volunteers discontinued from the study. A study done by Bhowik et al.[30] warns for the turmeric uses in people with bile duct obstruction, gallstones as excessive dosage could cause ulcers or cancer and reduce the number of red and white blood cells and also hair fall.

Considering the above mentioned side effects related to turmeric use in not only the physical appearance of the person and also the resulting digestive disorders, one will question themselves whether to use turmeric for treatment purpose or not.

Though there are numbers of articles that emphasize

on wide spectrum of activities of curcumin, this study did not find the antibacterial activity of turmeric. This could be because the curcumin might not have been extracted in significant amounts as the proper setup for its extraction was not available.

Though the present method of susceptibility by the authors did not show any activity of turmeric against the *S. aureus* ATCC 25923 strain, other studies following different methods for the extraction and sensitivity test has shown antibacterial activity.

CONCLUSION

Though turmeric was extracted using different solvents, yet, turmeric discs and different concentrations of turmeric extracts were ineffective against all the tested *E. coli* and *S. aureus* isolates. Thus, further research should be done on its extraction procedures, mechanism of action, bioactivity, and then only developing it as a therapeutic option should be thought of.

CONFLICT OF INTEREST

None declared.

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