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Research Article

# Towards Evading Antibiotic Resistance in Urinary Tract Infections: An Innovative Solution Through Non-Traditional Approach

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## Abstract

Burgeoning interest have been directed in the recent past towards antibiotic resistance exhibited by bacterial infections. Currently, global unrestrained use of broad-spectrum antibiotics has been singled out as the prime factor for this devastating issue. Therefore, any efforts to mitigate usage of antibiotics in most common recurrent bacterial infections such as urinary and respiratory tract infections could be considered as a boon to circumvent this global challenge. In this article, we report the novel curcumin formulation composed of potassium citrate as urinary alkaliser along with bioavailable modified curcumin extract as the natural antibacterial agent. The *in vitro* evaluation of this unique composition proved more than log 5 reduction of *E. coli* viable bacterial colonies belonging to strain from ATCC25922 with 455µg/mL as the minimum bactericidal concentration (MBC). It was also established that the presence of potassium citrate in the composition does not alter the antibacterial efficacy of the bioavailable modified turmeric extract.

## Abbreviations (if used)

a. Escherichia coli - E. Coli
b. Urinary Tract Infection (UTI)
c. Minimum Bactericidal Concentration - MBC

d. Minimum Inhibitory Concentration - MIC
e. Uropathogenic Escherichia coli - UPEC
f. Exopolysaccharides (EPS)

## Introduction

The emerging trend of antibiotic resistance and efforts to circumvent this growing global challenge will soon become the need of the hour.

Antibiotics are a class of medicine that treats and prevents bacterial infections. The coveted yet accidental discovery of Sir Alexander Fleming gave birth to the world's first ever antibiotic, namely the "Penicillin" [1].

Ever since the successful treatment of Streptococus septicemea by penicillin, its discovery has led to easy treatment of fatal diseases, such as bacterial endocarditis, bacterial meningitis and pneumococcal pneumonia. More specifically, the impact on replacement of antisera and sulfonamides for the treatment of pneumonia rapidly paved way for screening of other natural products [2], which has led to a myriad of treatment options involving different antibiotics for several bacterial infections.

However, currently, the usage of antibiotics, or more specifically excessive usage of antibiotics, has opened doors for a cluster of issues termed as antibiotic resistance. The resistance to antibiotics occurs when bacteria changes its response to a specific antibiotic. A major cause of the issue is by actions of some physicians who throw a broad spectrum of antibiotics to combat a specific disease due to impracticality in identifying the infectious agent and prescribing an appropriate antibiotic within a day [2]. The approach has its pros and cons. Yes, while it saves lives, it also leads to cluster of negative issues. Antibiotic resistance not only results in higher medical costs and prolonged hospital stays, but also increased mortality as previously benign infections are turning to be malicious.

A plausible solution is to reduce the time between prevention of illness and providing an appropriate treatment. This could potentially lead to reduction in resistance and unnecessary prescription, perhaps provision of a chance of speedy recovery for needy patients. The methodology could be applied in the case of most common bacterial infections such as bacteriuria, which leads to the discussion on urinary tract infections.

### Epidemiology of UTIs

Urinary tract infections are the most common form of bacterial infections. Globally, more than 150 million people annually affected [3]. Clinically, UTIs are classified as uncomplicated and complicated UTIs. Uncomplicated UTIs are prevalent in individuals who are otherwise healthy and have no structural or neurological abnormalities in the Urinary tract, whereas complicated UTIs arises due to mitigation of host defence mechanisms. Healthcare associated UTIs (HAUTI) and catheter associated UTIs are two major categories of complicated UTI. Uncomplicated UTs are further classified as lower UTIs (cystitis) and upper UTIs (pyelonephritis). Acute cystitis is the most common form of UTI in comparison to Acute pyelonephritis, justified by the occurrence of 18-24 episodes of the acute cystitis per episode of pyelonephritis

[3]. Furthermore, gender wise influence is demonstrated by most common occurrence of uncomplicated cystitis in women in comparison to men where complicated forms of UTI are prevalent.

UTIs can be caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTI is the uropathogenic Escherichia coli (UPEC) [4]. The prevalence of UPEC in uncomplicated cystitis is followed by Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis, group B Streptococcus (GBS) and Proteus mirabilis (Fig. 1).

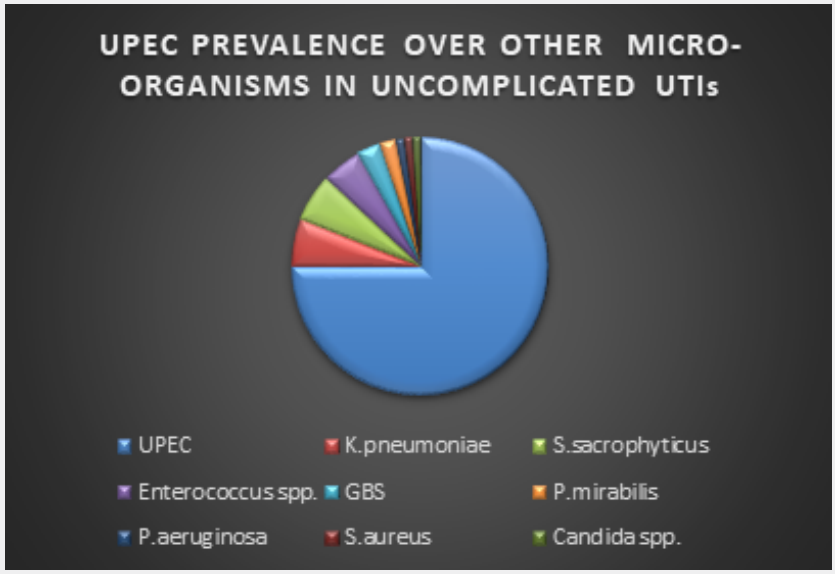


***Figure 1:*** *Depicting the Epidemiology of UTIs*

**UTI Pathogenesis**

Most typically, UTIs are initiated with periurethral contamination by a UPEC residing in the gut and subsequent colonization of the urethra and will be followed by migration of the pathogen to the bladder, an event that requires appendages such as flagella and pili. In the bladder, the consequences of complex host-pathogen interactions ultimately determine whether UPEC will be successful in colonization or eliminated. Multiple bacterial adhesins recognize receptors on the bladder epithelium (i.e. uroepithelium) mediate colonization [5]. Ascension does not halt there, indeed through multiplication and by overcoming host immune surveillance the uropathogens can subsequently ascend to the kidneys, wherein they colonize the renal epithelium. Furthermore, uropathogens may cross the tubular epithelial barrier to reach the blood stream, culminating in bacteraemia [5].

UPEC colonization during Uncomplicated urinary tract infection (UTI) is characterized by dysuria (pain during urination) and urinary frequency (very frequent urination and uncontrollable urge to void the

bladder) [6]. Most common intervention for patients suffering from symptomatic uncomplicated UTI is the antibiotics. However, long term use of such antibiotic treatment option inevitably leads to long-term alteration of the normal micro-biome residing in vagina, gastro-intestinal tract and may even lead to multi drug resistant bacterial strains. The development of resistance mainly prevalent in extended spectrum beta lactamase secreting enzymes.

Currently, antibiotics such as trimethoprim sulfamethoxazole, ciprofloxacin and ampicillin are the most commonly recommended therapeutics for UTIs [7]. However, continuous rise in antibiotic resistance (Fig. 2) and high recurrence rates virtually left us with very few effective first-line treatment options, such as cephalosporin and carbapenems [8].

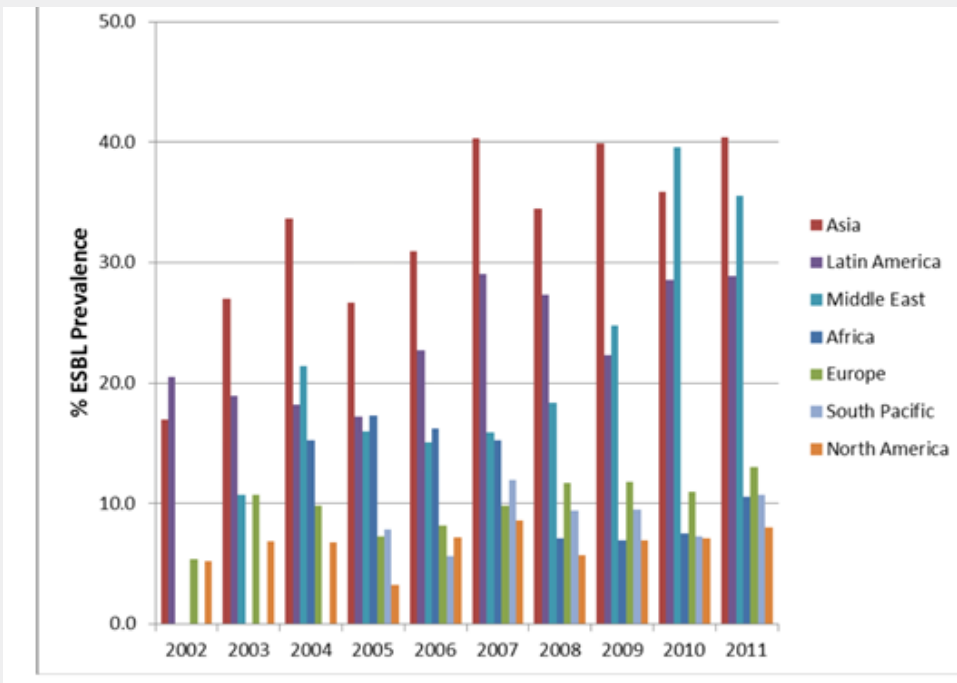


***Figure 2:*** *Prevalence of ESBLs in E. coli, K. pneumonia, K. oxytoca and P. mirabilis from urinary-tract infections by region from SMART 2009 to 2011 [7]*

However, with the knowledge of basic biology of UTI pathogenesis to specifically target virulence pathways, many promising approaches are being developed. One could envisage these anti-virulence therapeutics should theoretically enable us to effectively neutralize, or 'disarm', the capacity of UTI pathogens to cause disease, without altering the gut commensal microbiome.

Furthermore, majority of the therapeutic or prophylactic interventions are broadly based on two-faced strategy, namely, disperse the preformed biofilm and/or inhibit the formation of new biofilm [9]. The strategy could potentially provide solution for antibiotic resistance by reduction in duration between

prevention of illness and providing an appropriate treatment. This could potentially lead to reduction in resistance and unnecessary prescription, perhaps provision of a chance of speedy recovery for needy patients.

As the focus of the article is not on urinary tract infections, but rather an introduction to a plausible solution for it, interested readers are requested to read elsewhere. Briefly, urinary tract infections (lower region) referred to infection of the bladder and the urethra, known as cystitis and urethritis, respectively.

*E. coli* bacterial transfer from faecal residue through the rectum to bladder has been facilitated by the proximity of rectum and bladder in women. These infections yield an acidic urine which leads to a burning sensation during urination. The most common approach is to provide symptomatic relief by giving potassium citrate [10]. Potassium citrate alkalize the urine by enhancing plasma bicarbonate concentration due to its metabolic conversion *in vivo.*

**Development of Antibiofilm Agents**

As the central science, Chemistry equally plays a major role in the development of antibiofilm agents in conjunction with genetic engineering. A number of chemical moieties including [11]. In order to provide a glimpse on the mechanism of preventing biofilm formation, it is necessary to understand the structure of a biofilm. The matrix component of a biofilm consists of exopolysaccharides, or some time known as exopolymeric substances (EPS), are secreted by bacteria to their surroundings. These exopolymeric substances promotes adhesion and colonisation of bacterial cells. EPS, therefore, acts as a safety net for the growth and activity of bacterial cells within a biofilm.

One could envisage biomimetic supramolecular designs that prevent adhesion could be a good starting point. Indeed, several successful *in vitro* and *in vivo* studies have been reported on this supramolecular chemistry approach [12].

In fact, resolving antibiotic resistance caused by biofilm formation could be approached from many angles and supramolecular chemistry approach is just only one effective way of resolving the issue.

Along these lines, therapeutic interventions based on natural and synthetic origin have been evaluated as alternative therapies to antibiotics. More specifically flavonoids, a class of natural product has been widely investigated [13]. Infact the current treatment for uncomplicated lower UTIs based on Cranberries is founded on proanthocyanidins, a class of flavonoids acting against adhesion of bacterial cells and thereby preventing biofilm formation to some extent. Furthermore, therapeutic interventions based on cranberries and other similar treatment options provide only prophylactic benefit by providing symptomatic relief. Moreover, they hardly provide a lasting solution for recurrence of uncomplicated UTIs.

Taking in to consideration of above facts, investigators of Neptune Bio-innovations (NBI), Australia, envisaged that if they could find a natural flavonoid equivalent that could both destroy the preformed biofilm while acting as a "firewall" for new biofilm formation would then provide a solid foundation towards more lasting solution for patients undergoing UTI discomfort. Prompted by the marketing hype on turmeric and also backed by literature evidence [14], investigators of NBI focused their attention on curcuminoids, a class

of polyphenolic compounds and initially investigated its antibactericidal properties by utilising turbidity assays based on minimum inhibitory concentration based on standard protocols.

The pre-liminary research gave confidence and inspiration to dive deeper, more specifically to carry out minimum bactericidal concentration (MBC) assays, the result was the astonishing finding that curcumins able to provide greater than log 5 reduction of bacterial colonies with just 0.016uM concentration, as obtained from regression analysis of the assay. Its noteworthy that presence of greater than 100,000 colonies per milliliter of urine is reported as significant bacteriuria [15].

## Materials and Methods

**Objective:** To determine the IC50 of UC-IC50-2 sample supplied by Neptune Bio-Innovations against E. coli (ATCC 29522) via BSEN 1276:2009 Chemical disinfectants and antiseptics

**Scope of BSEN 1276:** The BSEN 1276 specifies a test method and the minimum requirements for bactericidal activity of chemical disinfectant and antiseptic products that form a homogeneous, physically stable preparation when diluted with hard water or - in the case of ready-to-use products - with water.

**Methodology and Appropriate Modification:** BSEN 1276:2009 tested against 10 concentrations. The protocol was modified to start with a 200% stock solution.

### Materials and Methods

Distilled water
Tryptone Soya Agar (TSA) Oxoid CM131
0.1% Peptone
Nutrient Broth 2.2% Lecithin & 3% tween
Standard Hard Water

### Equipment

Ratek VM 1 vortex 2700rpm
Preparation of Standard Hard Water

### General Procedure

Each of the prepared dilutions was pre-warmed in a 37°C incubator for 30 minutes until inoculation of the culture. Each dilution was inoculated with 0.05ml of *E. coli* ATCC 25922 prepared at a concentration of $5x10^9$cfu/ml and vortexed for 10 seconds at 2,700rpm. The inoculated samples were then placed back into the 37°C incubator for 24 hours contact time. At the end of the 24-hour incubation, 4.5ml of NBLTW neutralizer was added to each test matrix and vortexed for 10 seconds at 2,700rpm. This was considered the $10^{-1}$ dilution.

Serial dilutions of the neutralized matrix were prepared by taking 1ml of the neutralized sample and dispensing into 9ml of 0.1% peptone water to obtain the $10^{-2}$ dilution. This process was repeated another three times to obtain dilutions from $10^{-1}$ to $10^{-5}$.

From each of the prepared dilutions 2ml was taken and plated 1ml in duplicate for each dilution in sterile labelled petri dishes. The plates were then poured with 15ml of TSA.

The plates were dried for 15 minutes and incubated at 37˚C for 48 hours.

**Solution A:**

Dissolve 19.84g magnesium chloride ($MgCl_2$) and 46.24g calcium chloride ($CaCl_2$) in water and dilute to 1000ml. Sterilize in the autoclave.

**Solution B:**

Dissolve 35.02g sodium bicarbonate ($NaHCO_3$) in water and dilute to 1000ml. Sterilize by membrane filtration.

**BSEN Standard Hard Water:**

Placed approximately 650mL of BSEN-SHW in a 1000mL volumetric flask and add 6,0mL of solution A, then 8,0ml of solution B. Mix and dilute to 1000mL with water. The pH of the hard water shall be 7,0 ± 0,2, when measured at (20 ± 1)˚C as per BSEN and the pH was adjusted accordingly, using 1M HCl or 1M NaOH.

**Preparation of Nutrient Broth 2.2% Lecithin & 3% tween**

25g of Oxoid Nutrient Broth 2 Powder Base (CM67) with the addition of 130g of Lecithin and 30g of Tween 80 was made up to 1000ml with distilled water. Dispensed into Macs and then autoclaved at 121˚C for 15 minutes.

**Preparation of E. coli ATCC 25922 Suspension**

Colonies are taken from a plate prepared with *E. coli* and placed into 10ml of 0.1% Peptone containing glass beads. Enough colonies were taken to produce a culture in the range of $5^{-10}x10^{9}$cfu/ml.

**Preparation of Stock solution of UC-IC50-2**

UC-IC50-2 was prepared by blending modified bioavailable turmeric extract and potassium citrate monohydrate in a certain ratio.

**UC-IC50-2-Stock A (200% Stock Solution) -2g/mL**

Accurately weighed 3.00g of UC-IC50-2 was added to an Eppendorf labelled as UC-IC50-2-Stock A and was dissolved in 1.50mL of BSEN Standard Hard Water (BSEN-SHW) as per the instructions provided by Neptune Bio-innovations and vortexed until completely dissolved.

**Preparation of Stock Dilutions of UC-IC50-2**

The 200% stock solution was then used to prepare the following serial dilutions:

**Preparation of Serial Dilutions**

***UC-IC50-2-WSB_100 (100% Solution) - 1g/mL***

0.25ml of UC-IC50-2-Stock A was diluted with 0.25ml of BSEN SHW and vortexed for 10 seconds at 2,700rpm to produce UC-IC50-2-Working Stock B (100%) and labelled as UC-IC50-2-WSB_100.

***UC-IC50-2-WSB_90 (90% solution) - 0.9g/mL***

0.225ml of the UC-IC50-2- Stock A solution was diluted with 0.275ml of BSEN SHW and vortexed for 10 seconds at 2,700rpm to produce UC-IC50-2-Working Stock B (90%) and labelled as UC-IC50-2-WSB_90.

***UC-IC50-2-WSB_80 (80% solution) - 0.8g/mL***

0.200ml of the UC-IC50-2-Stock A solution was diluted with 0.300 ml of BSEN SHW and vortexed for 10 seconds at 2,700rpm to produce UC-IC50-2-Working Stock B (80%) and labelled as UC-IC50-2-WSB_80.

***UC-IC50-2-WSB_70 (70% solution) - 0.7g/mL***

0.175 ml of the UC-IC50-2-Stock A solution was diluted with 0.325 ml of BSEN SHW and vortexed for 10 seconds at 2,700rpm to produce UC-IC50-2-Working Stock B (70%) and labelled as UC-IC50-2-WSB_70.

***UC-IC50-2-WSB 60 (60% solution) - 0.6g/mL***

0.15 ml of the UC-IC50-2-Stock A solution was diluted with 0.350 ml of BSEN SHW and vortexed for 10 seconds at 2,700rpm to produce UC-IC50-2-Working Stock B (60%) and labelled as UC-IC50-2-WSB_60.

***UC-IC50-2-WSB 50 (50% solution) - 0.5g/mL***

0.125 ml of the UC-IC50-2-Stock A solution was diluted with 0.375 ml of BSEN SHW and vortexed for 10 seconds at 2,700rpm to produce UC-IC50-2-Working Stock B (50%) and labelled as UC-IC50-2-WSB_50.

***UC-IC50-2-WSB 40 (40% solution) - 0.4g/mL***

0.100 ml of the UC-IC50-2-Stock A solution was diluted with 0.400 ml of BSEN SHW and vortexed for 10 seconds at 2,700rpm to produce UC-IC50-2-Working Stock B (40%) and labelled as UC-IC50-2-WSB_40.

***UC-IC50-2-WSB 30 (30% solution) - 0.3g/mL***

0.075ml of the UC-IC50-2-Stock A solution was diluted with 0.425ml of BSEN SHW and vortexed for 10 seconds at 2,700rpm to produce UC-IC50-2-Working Stock B (30%) and labelled as UC-IC50-2-WSB_30.

***UC-IC50-2-WSB 20 (20% solution) - 0.2g/mL***

0.050ml of the UC-IC50-2-Stock A solution was diluted with 0.450ml of BSEN SHW and vortexed for 10 seconds at 2,700rpm to produce UC-IC50-2-Working Stock B (20%) and labelled as UC-IC50-2-WSB_20.

***UC-IC50-2-WSB 10 (10% solution) - 0.1g/mL***

0.025ml of the UC-IC50-2-Stock A solution was diluted with 0.475ml of BSEN SHW and vortexed for 10 seconds at 2,700rpm to produce UC-IC50-2-Working Stock B (10%) and labelled as UC-IC50-2-WSB_10.

## Results

***Table 1:*** *Results used to determine dose response curve for UC-IC50-2*

| UC-IC50-2 Concentration (g/mL) | Average of Initial concern | Average of Initial concern | Initial Concentration Data |
|---|---|---|---|
| | E.Col_Replicate 1 (cfu/mL) | E.Col_Replicate 2 (cfu/mL) | Average (cfu/mL) |
| 1.0g/mL | 2.4E+07 | 2.4E+07 | 2.4E+07 |
| 0.9g/mL | 2.4E+07 | 2.4E+07 | 2.4E+07 |
| 0.8g/mL | 2.4E+07 | 2.4E+07 | 2.4E+07 |
| 0.7g/mL | 2.4E+07 | 2.4E+07 | 2.4E+07 |
| 0.6g/mL | 2.4E+07 | 2.4E+07 | 2.4E+07 |
| 0.5g/mL | 2.4E+07 | 2.4E+07 | 2.4E+07 |
| 0.4g/mL | 2.4E+07 | 2.4E+07 | 2.4E+07 |
| 0.3g/mL | 2.4E+07 | 2.4E+07 | 2.4E+07 |
| 0.2g/mL | 2.4E+07 | 2.4E+07 | 2.4E+07 |
| 0.1g/mL | 2.4E+07 | 2.4E+07 | 2.4E+07 |

| UC-IC50-2 Concentration (%) | Average of final concentration 24 Hours | Average of final concentration 24 Hours | Final concentration Data 24 Hours |
|---|---|---|---|
| | E.Col_Replicate 1 (cfu/mL) | E.Col_Replicate 2 (cfu/mL) | Average (cfu/mL) |
| 0.1g/mL | 139 | 139 | 139 |
| 0.2g/mL | 139 | 139 | 139 |
| 0.3g/mL | 139 | 139 | 139 |
| 0.4g/mL | 139 | 139 | 139 |
| 0.5g/mL | 139 | 139 | 139 |
| 0.6g/mL | 139 | 139 | 139 |
| 0.7g/mL | 139 | 139 | 139 |
| 0.8g/mL | 2.0E+02 | 2.4E+02 | 2.2E+02 |
| 0.9g/mL | 5.3E+05 | 5.3E+05 | 5.3E+05 |
| 1.0g/mL | 2.3E+07 | 2.4E+07 | 2.4E+07 |

| UC-IC50-2 Concentration (%) | Initial concentration Data | Final concentration Data 24 Hours | Percentage cell viability |
|---|---|---|---|
| | Average (cfu/mL) | Average (cfu/mL) | 24 hours |
| 0.1g/mL | 2.4E+07 | 139 | 99.999 |
| 0.2g/mL | 2.4E+07 | 139 | 99.999 |
| 0.3g/mL | 2.4E+07 | 139 | 99.999 |
| 0.4g/mL | 2.4E+07 | 139 | 99.999 |
| 0.5g/mL | 2.4E+07 | 139 | 99.999 |
| 0.6g/mL | 2.4E+07 | 139 | 99.999 |
| 0.7g/mL | 2.4E+07 | 139 | 99.999 |
| 0.8g/mL | 2.4E+07 | 2.2E+02 | 99.999 |
| 0.9g/mL | 2.4E+07 | 5.3E+05 | 97.809 |
| 1.0g/mL | 2.4E+07 | 2.4E+07 | 1.948 |

## Discussions

A wide variety of methods have been in practice to evaluate the efficacy of antimicrobial activity of medicinal herbal extracts, agar-disk diffusion, ATP bioluminescence agar or broth dilution to name but a few [16]. However, dilution methods based on agar or broth are effective both in their efficacy to evaluate minimum inhibitory concentration (MIC) of the antibacterial agent as well as from cost perspective. Indeed, broth micro or macro dilution methods are most widely practiced antimicrobial susceptibility testing. However, inoculum size, type of growth medium and incubation time and diversity in the preparation method could influence MIC values.

The minimum inhibitory concentration (MIC value) recorded is defined as the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth of the microorganism in question. Wherein, minimum bactericidal concentration (MBC) is defined as the lowest concentration of the assayed antimicrobial agent that is required to kill 99.9% of the specific bacterial strain. A series of dilutions of UC-IC50-2 was prepared and was subjected antibacterial kill rate assays as described in the preceding section. A precise evaluation of MBC was achieved by obtaining Dose-Response curve using GraphPad-Prism.

Furthermore, minimum bactericidal concentration of 455µg/ml obtained in this study is in line with the investigative reports published elsewhere [16]. However, antibacterial properties of *Curcuma longa* in its native form or as derivatives in conjunction with potassium citrate in the same formulation has never been explored. Furthermore, antibacterial properties of curcuminoids has not been affected by the presence of alkaliser. Ten serial dilutions have been considered to ensure that there are enough assay concentrations having responses on the lower and upper plateaus of the dose-response curve (Fig. 3).

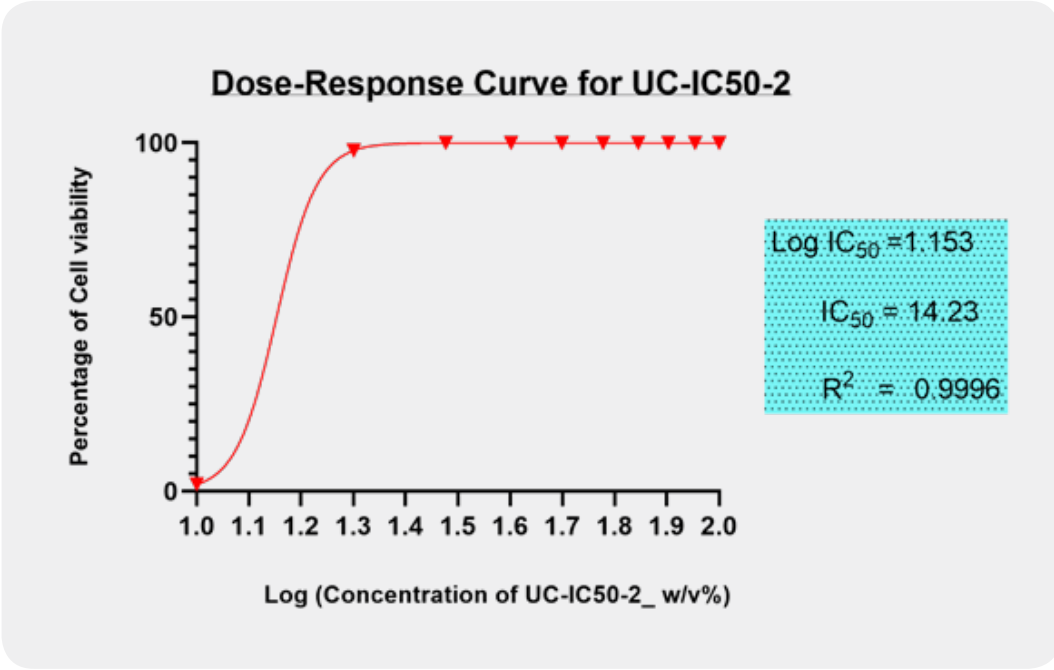


***Figure 3:*** *Dose-Response curve decipting the lowest concentration required of UC-IC50-2 to kill more than 50% of E. Coli*

Therefore, we have been prompted to opt out a standardized protocol established by clinical and laboratory standards institute (CLSI) or BESN. The results have been investigated independently from three laboratories and were confirmed with similar results with same standardized protocols.

Essentially, there are other bacterial strains have been identified as causative agents for UTIs [4]. Although this study had focused on the most probable bacterial strain causing uncomplicated UTI, namely ATCC25922, it will be equally interesting to investigate other types of bacterial strains and related therapeutic potential of

this formulation. Moreover, the therapeutic efficacy of this formulation could be investigated for other type of non-serious diseases caused by ATCC25922.

## Conclusions

We observed reduction of greater than log 5 in the viable E. Coli colonies with just 0.016 micro molar concentration of the active, obtained from regression analysis of the assay. The investigators of NBI strongly believe that the mechanism of action is based on "apoptosis like" killing of bacteria. Currently, the hypothesis has been well established as a scientific fact through an in-depth study published in PLOS One, utilizing the specific strain used in NBI investigator's assay [17]. On the other hand, incorporation of potassium citrate along with curcumins (provided from a modified bioavailable turmeric extract) does not prevent the antibactericidal property of curcumins and does not contribute to any pH variation whatsoever in solution, as proved in long term stability studies though clinical therapeutic efficacy is yet to be established.

## Bibliography

1. Tan, S. Y. & Tatsumura, Y. (2015). Alexander Fleming (1881-1955): Discoverer of penicillin. *Singapore Med J., 56,* 366-367.

2. Maxson, T. & Mitchell, D. A. (2016). Targeted Treatment for Bacterial Infections: Prospects for Pathogen-Specific Antibiotics Coupled with Rapid Diagnostics. *Tetrahedron, 72,* 3609-3624.

3. Kranz, J., Schmidt, S., Lebert, C., Schneidewind, L., Mandraka, F., Kunze, M., *et al.* (2018). The 2017 Update of the German Clinical Guideline on Epidemiology, Diagnostics, Therapy, Prevention, and Management of Uncomplicated Urinary Tract Infections in Adult Patients. *Part II: Therapy and Prevention, Urol. Int., 100,* 271-278.

4. Terlizzi, M. E., Gribaudo, G. & Maffei, M. E. (2017). UroPathogenic Escherichia coli (UPEC) Infections: Virulence Factors, Bladder Responses, Antibiotic, and Non-antibiotic Antimicrobial Strategies. *Front Microbiol.*

5. Abraham, S. N. & Miao, Y. (2015). The nature of immune responses to urinary tract infections. *Nature Reviews Immunology, 15,* 655-663.

6. Wrenn, K. (1990). In Clinical Methods: The History, Physical, and Laboratory Examinations, eds. H. K. Walker, W. D. Hall and J. W. Hurst, Butterworths, Boston, 3rd edn., 1990.

7. Lashkar, M. O. & Nahata, M. C. (2018). Antimicrobial Pharmacotherapy Management of Urinary Tract Infections in Pediatric Patients. *J Pharm Technol., 34,* 62-81.

8. David Paterson, L. & Robert Bonomo, A. (2019). Extended-Spectrum β-Lactamases: a Clinical Update. *Clinical Microbiology Reviews*, 657-686.

9. Roy, R., Tiwari, M., Donelli, G. & Tiwari, V. (2017). Strategies for combating bacterial biofilms: A focus on anti-biofilm agents and their mechanisms of action. *Virulence, 9,* 522-554.

10. Beerepoot, M. & Geerlings, S. (2016). Non-Antibiotic Prophylaxis for Urinary Tract Infections. *Pathogens, 5,* 36.

11. Rabin, N., Zheng, Y., Opoku-Temeng, C., Du, Y., Bonsu, E. & Sintim, H. O. (2015). Agents that inhibit bacterial biofilm formation. *Future Medicinal Chemistry, 7,* 647-671.

12. Li, X., Wu, B., Chen, H., Nan, K., Jin, Y., Sun, L. & Wang, B. (2018). Recent developments in smart antibacterial surfaces to inhibit biofilm formation and bacterial infections. *J. Mater. Chem. B., 6,* 4274-4292.

13. Beerepoot, M. A. J., ter Riet, G., Nys, S., van der Wal, W. M., de Borgie, C. A. J. M., de Reijke, T. M., *et al.* (2011). Cranberries vs Antibiotics to Prevent Urinary Tract Infections: A Randomized Double-blind Noninferiority Trial in Premenopausal Women. *Arch Intern Med., 171,* 1270-1278.

14. Afrose, R., Saha, S. K., Banu, L. A., Ahmed, A. U., Shahidullah, A. S., Gani, A., *et al.* (2015). Antibacterial Effect of Curcuma longa (Turmeric) Against Staphylococcus aureus and Escherichia coli. *Mymensingh Med J., 24,* 506-515.

15. Orenstein, R. & Wong, E. S. (1999). Urinary Tract Infections in Adults. *AFP, 59*(5), 1225-1234.

16. Gunes, H., Gulen, D., Mutlu, R., Gumus, A., Tas, T. & Topkaya, A. E. (2016). Antibacterial effects of curcumin: An *in vitro* minimum inhibitory concentration study. *Toxicol Ind Health, 32,* 246-250.

17. Tyagi, P., Singh, M., Kumari, H., Kumari, A. & Mukhopadhyay, K. Bactericidal Activity of Curcumin I Is Associated with Damaging of Bacterial Membrane, *PLoS One.*