Invitro Antimicrobial Efficacy of *Jatropha Curcas* Extracts on *Escherichia Coli* from Diabetic Females in Kitui County Kenya

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Abstract

Background: Herbal usages for Urinary tract infections (UTI) in diabetes have been used for centuries in Kitui County. The plant *Jatropha curcas* is of numerous natural yields, traditional healers in Kitui County have been using various portions of the plant in different ways including bacterial infections treatment. Non-Communicable Diseases in Kenya gives 28% deaths where 2% was diabetes. Prevalence of diabetes in Kitui County is at 29. High glucose level in urine provides a conducive environment for pathogenic microorganisms *Escherichia coli* which is the utmost major cause of UTI at approximately 80% populating the colon.

Methods: This is an Experimental and Cross-sectional method of research, in which the *Escherichia coli* clinical isolates were obtained from diabetic females identified with UTI at Kitui County Referrals Hospital and transported to Mount Kenya University. Crude *Jatropha Curcas* leaves and stem barks were harvested from Kitui County and Phytochemistry analysis done at East African Herbarium in the National Museums of Kenya, then after were subjected to clinical isolates of *Escherichia coli* for antimicrobial efficacy by evaluating their Minimum Inhibition Concentration and Minimum Bactericidal Concentration at Mount Kenya University, Microbiology Laboratory.

Results: Minimum Inhibition Concentrations produced Negative results and thus Minimum Bactericidal Concentration procedure was not done. A prevalence rate of 69% of *Escherichia coli* isolates as the causative agent of UTIs in diabetic females of Kitui County, Kenya was noted.

Conclusion: *Jatropha curcas* is bacterial static against *E. coli* in Urinary Tract Infections of diabetic females located at Kitui County, Kenya

Keywords: *Jatropha curcas*, *Escherichia coli*, Urinary tract infection (UTIs), and Diabetes.
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1. Introduction

Non-Communicable Diseases in Kenya gives 28% deaths where 2% was diabetes. Prevalence of diabetes in Kenya approximates at 3.3% and is forecasted to escalate to 4.5% by 2025 [1]. Prevalence of diabetes is estimated to be 16 % in rural Kenya, and in Kitui County, is at 29.1% Disease and death in patients with diabetic is normally triggered by many infections including urinary tract infection which remains a burden in the diabetic community globally. High glucose level in urine provides a conducive environment for pathogenic microorganisms *Escherichia coli* which is the utmost major cause of Urinary tract infection at approximately 80% populating the colon [2,3].

Diabetes is metabolic condition that is characterized by increasing blood glucose. Disease and death in patients with diabetic are normally triggered by many infections including urinary tract infection which remains to be a burden in the diabetic community globally [4]. Patients with diabetes have malfunctioning bladder prompting urine buildup which serves as a conducive state to the bacteria to cultivate, grow and hence lead to urinary tract infection. High glucose level in urine provides a conducive environment for pathogenic microorganisms *Escherichia coli*. Urinary tract infection (UTI) is a conjoint disease in female diabetics due to their; anatomical small urethra, easy detoxification of urinary tract with fecal flora, previous history of UTI, parity, contraceptives use leading to hormonal changes, social-economic status including individual hygiene and changes in urine chemistry where elevated glucose facilitates bacterial growth. [4,5]. Continued allopathic treatment with various antibiotics is known to advance resistance to the existing antibiotics that have been prescribed, if the medication is used for a long time or frequently, an issue which has led to a continuous investigation of different modes of treatment of UTI in diabetic female patients and substitute therapies [6,7]. *Escherichia coli* [6] is the utmost major cause of UTIs at approximately 80% populating the colon [2].

Herbal usages for UTIs in diabetes have been used for centuries in Kitui County. Herbal therapies may relieve urinary tract infections through combating the *Escherichia coli* bacteria, diminishing irritation and curing urinary tract tissues and hence help in the prevention of future occurrences [6]. *Jatropha curcas*, belongs to the family Euphorbiaceae. The variety name *Jatropha* is a consequence of two Greek words jatr'os (specialist) and troh'e (food), demonstrating its valuable use in conventional medication [8]. It is accepted to have begun from Mexico and northern Focal America on the grounds that in these areas, the most noteworthy hereditary variety was found [9,10]. The plant *Jatropha curcas* is of numerous natural yields, maximum of which have been broadly of value to human health in the management of numerous health conditions [8,10]. The traditional healers use various portions of

the plant: leaves, branches, fruits, seeds, latex, stem bark, twigs, and roots in different ways including bacterial infections treatment in traditional folk medicine [7]. For antibiotic resistance befalls as part of a natural evolution course, where some are static unlike others which are cidal to bacteria, thus different antibiotics will be necessary to keep up with resistant bacteria [5]. This experimental study was intended at assessing the antimicrobial efficacy of *Jatropha curcas* leaves and stem bark extracts, singly on *Escherichia coli*, a known gram-negative bacterium and a common contributing instrument of urinary tract infection (UTI) in diabetic females attending Kitui County hospital, in Kitui County, Kenya.

2. Materials and methods

Plant material collection

Fresh young crude *Jatropha curcas* leaves and stem barks were harvested from Kitui County. The two plant parts were collected and ferried to East African Herbarium in the National Museums of Kenya for extraction and phytochemical analysis. Voucher specimens (NMK/BOT/CTX/1/2) were store as it is indicated by [11].

Clinical samples

Fasting blood sugar test

Fasting blood sugar testing (FBS) an indicator for people with diabetes was used to help assess how well diabetes is being managed via Finger prick to obtain a drop of blood for purposes of the test.

Urine sample

Mid - stream urine (MSU) was used for culture to investigate the presence of *Escherichia coli* in the urinary tract. A MSU was collected into a sterile bottle. Patients was informed to pass a slight amount of urine into the toilet or latrine to certify that bacteria, cells or parasites that have entered the lower urethra from the vagina or perennial area are flushed away, and then collect about 20ml of urine into the sterile bottle, the remaining urine in the bladder is passed. Macroscopy and Microcopy diagnosis was performed, then by a dipstick deliberated to identify urine nitrite and to indirectly estimate the number of segmented neutrophils.

Culturing urine sample

Urine sample was mixed to re-suspend existing microorganism. A 10 µl standardized loop was dished in upright position in the urine and removed, the collected fluid was inoculated in Nutrient and MacConkey agars respectively. The respective inoculated Medias was incubated for an overnight at an optimum growth temperature of 37°C.

Quality Controls

The culture media was incubated less of any isolates thus testing its sterility. After preparation, inoculation with known *Escherichia coli ATCC 29218* isolates, was done to confirm support growth. Positive control drug and Negative control drug was used in support of control measures.

Anti-bacterial assay

Escherichia coli clinical isolates was suspended in glycerol. The Mueller Hinton agar was set then dispersed in petri dishes in which the clinical isolates was inoculated and incubated overnight at 37°C to enable recovery of micro-organism.

Preparation of media.

A. Preparation Mueller Hinton agar.

Mueller-Hinton agar (MHA), was the paramount medium to use for routine susceptibility testing for non-fastidious bacteria. It is also the most acknowledged medium used for most broth dilution testing as the condition contents are well preserved.

B. Preparation McFarland turbidity standard.

The adjustment of the turbidity and concentration of the inoculums will use McFarland turbidity standard preparation when performing antimicrobial susceptibility test.

The diffusion method of Kirby Bauer disc. A modified diffusion technique of Kirby-Bauer disc for Antimicrobial Susceptibility Testing by measuring zones of inhibition was applied. Gray shading indicated a confluent lawn of bacterial growth while the white circle indicated absence of growth of the test organisms.

Measurement of Inhibition Zones

300 mg of every unrefined plant separate was broken down in 1000 µl (1ml) of DMSO. A stock miniature weakening technique was followed to decide the base inhibitory Focus for the dynamic rough concentrates against *E. coli* consulting with the Clinical Standard Establishment. The tests were acted in 96 well miniature titer plates. Sequential multiplying weakening's were acted in which the succeeding fixation all around was half of the focus in the past well. The MIC was resolved just where the plant remove areas of strength for introduced action (≥ 9 mm) by the circle dispersion technique. The wells were loaded up with 50 µl of the Muller Hinton stock for bacterial strains. Then, 50 µl of the plant extract (made by dissolving 300 mg of each concentrate in 1000 µl (1ml) of DMSO for complete disintegration) were administered into the principal a long time before sequential weakening's. The sequential weakening's were achieved by moving 50 µl of Muller Hinton stock including the concentrate from the initial well through the second, third and fourth wells. Then 50 µl of the test disconnects were dispersed into each well. One line of wells was utilized as negative control of the development of the microorganisms in the medium, while 50 µl of the anti-microbial ciprofloxacin were utilized as a positive control. Miniature titer plates were covered. Microbes were brooded at 37 ºC for 24 hrs.

Inhibition zones measurements and interpretation after incubation time was to determine the antimicrobial potential of *Jatropha Curcas* crude plant extracts leaves and stem bark s against *E.coli*, designated by clear zones of growth inhibition around each disc diameter and was documented in millimeters by use of a ruler on the undersurface of the plate minus opening the lid. Inhibition diameter zones was dignified by the boundaries of the dense growth. The inhibition zones were compared with the zone-size interpretation of acknowledged growth inhibition zones and documented as susceptible, intermediate or resistant to each crude drug [12].

Minimum Inhibition Concentration and Minimum Bactericidal Concentration

The determination of the MIC value using the REMA was based on the color change indicator as a result of growing bacteria interaction with the indicator, known to change from blue to pink purple in presence of bacteria growth [13,12].

Culture methods

The test organic entities were put on Supplement Agar (NA) slants, ready from Supplement Stock base at 2·5 °C, all miniature creatures utilized in MIC measures were two times passed on 16-18 h societies filled in Supplement Stock.)

Assay media

Through the resazurin MIC strategy (REMA), the inoculum was weakened to the suitable cell densities in NB containing 0·15% (w/v) agar for all test societies for which BHIB with 0·15% (w/v) agar was utilized. Before vaccination, test media were liquefied by steaming and tempered to 37 °C, at which temperature they stayed fluid [13].

Inoculum densities

The cell fixation expected to cause decrease of resazurin inside 2 not entirely set in stone for every one of the test creatures. Sequential 10-crease weakening's of each culture were ready.

NB. Aliquots (1·7 ml) were administered into tubes containing 0·2 ml 'messy' (0·15%, w/v) agar, and 0·1 ml resazurin arrangement. The cylinders were brooded for 2 h at 37 °C, at which point aliquots from adjoining blue (oxidized) mauve and pink (diminished) weakening cylinders were tried by the plate count strategy. Sequential twofold weakening's (0·005-1·25%, v/v) of the concentrates were ready by votexing in room-temperature. The resazurin examine medium was then vaccinated with the test organic entity to yield a last cell thickness expected to lessen resazurin. The inoculum thickness was affirmed by plate count. A clean 96-well microtiter plate with top was set up with every one of the test microorganisms. (n_8) as follows:

Column 1–9, 170 ml inoculum plus 20 ml of a plant extract;

Column 10, 170 ml inoculum plus 20 ml plant diluent (positive control);

Column 11 and 12, 170 ml sterile resazurin assay medium plus 20 ml plant diluent (Negative control and Blank, respectively).

Well items were totally blended utilizing the micropipette Two plate were ready for every organic entity and brooded at 37 °C for either 3·5 h or 18 h. After brooding, 10 ml of resazurin arrangement was added to all aside from segment 12, to which 10 ml of refined water was added. Following a second brooding of 2 h at 37 °C, wells were evaluated outwardly to decide the MIC values [13]. Values of MIC obtained determined the proceeding of MBC which was not done.

Validity and Reliability of the data

Validity will ensure precise presentation and elucidation of the results, this was ensured by use of acknowledged *Escherichia coli ATCC 29218* from Microbiology section, National public health reference laboratory, Nairobi as a control bacterium. Reliability of this study was the constancy of a degree that guarantees uniformity of this study. The consistency was confirmed by usage of the positive and negative control thus measuring both Minimum inhibition concentration and minimum bactericidal concentration.

3. Results

Susceptibility testing of Clinical isolates and Control bacteria

A total of 211 female Diabetic patients were engaged for the research as a sample size via testing their Fasting blood sugars. Urinalysis via Macroscopy, Dipstick and Microscopy was done to rule out Urinary tract infection followed by Culture in Cled media which confirmed 101 female diabetic patients to have UTIs of suspected *E. coli* growth via their morphological characteristics. Out of the 101 suspected *E. coli* clinical isolates, 70 confirmed to be true *E. coli* via the biochemical test use.

The findings indicated a Fasting blood sugar at age's 41-45years (table 1 and figure 2) to be correlating with high leucocytes frequency at the same age intervals and thus fasting blood sugar levels are directly proportional to leucocytes values.

Presumptive and Purification of the primary clinical isolates

Presumptive and Purification of the 101 primary isolates of *E.Coli* through biochemical tests gave a total of 70 pure *E.Coli* clinical isolates which were further pooled together for efficacy test with Jatropha curcas extracts with controls.

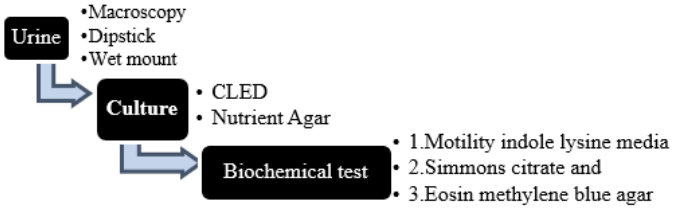

Figure 1. Info graph of *E. coli* analysis procedure.

Inhibition Zones

Antimicrobial activity of *Jatropha Curcas* extracts on methanol, acetone, aqueous and ethyl acetate solvents was determined against Clinical isolate *E. coli* and *E. coli* Control bacteria ATCC 29218. A presumptive piloting exercise of Jatropha *Curcas* leaves extracts inoculation on *E. coli* Inoculation in triplicate was done based on key solvents as reflected in table 2 and 3.

Minimum Inhibition Concentration

Double serial dilution of the extracts was done and the lowest concentration that inhibited growth was recoded in milligrams per milliliter. Minimum inhibitory concentration that was able to prevent growth of micro-organism. MIC 50 is the concentration that is able to inhibit growth of 50% of the micro-organism while MIC90 is the Minimum concentration that inhibits growth of 90% of the bacteria. The minimum inhibitory concentration of the plant extracts was determined by the broth micro dilution method in 96 well microtiter plates for MI. The determination of the MIC value was done using the Resazurin-based microplate assay (REMA) method [13], based on the change of color resulting from interaction between indicators with the growing test bacteria, changing from blue to pink purple in presence of bacterial growth. See tables 4a, 4b, 5a and 5b.

Table 1: Correlation of Fasting Blood Sugar with Urine glucose and leucocytes

Sugars &Urinalysis parameters totals	Ages (Yrs.)												
	21-25	26-30	31-35	36-40	41-45	46-50	51-55	56-60	61-65	66-70	71-75	76-80	81-85
FBS	2	5	8	11	16	14	11	10	9	7	5	2	1
Urine Glucose	2	5	8	11	16	14	11	10	9	7	5	2	1
Urine Leucocytes	2	5	8	11	16	14	11	10	9	7	5	2	1

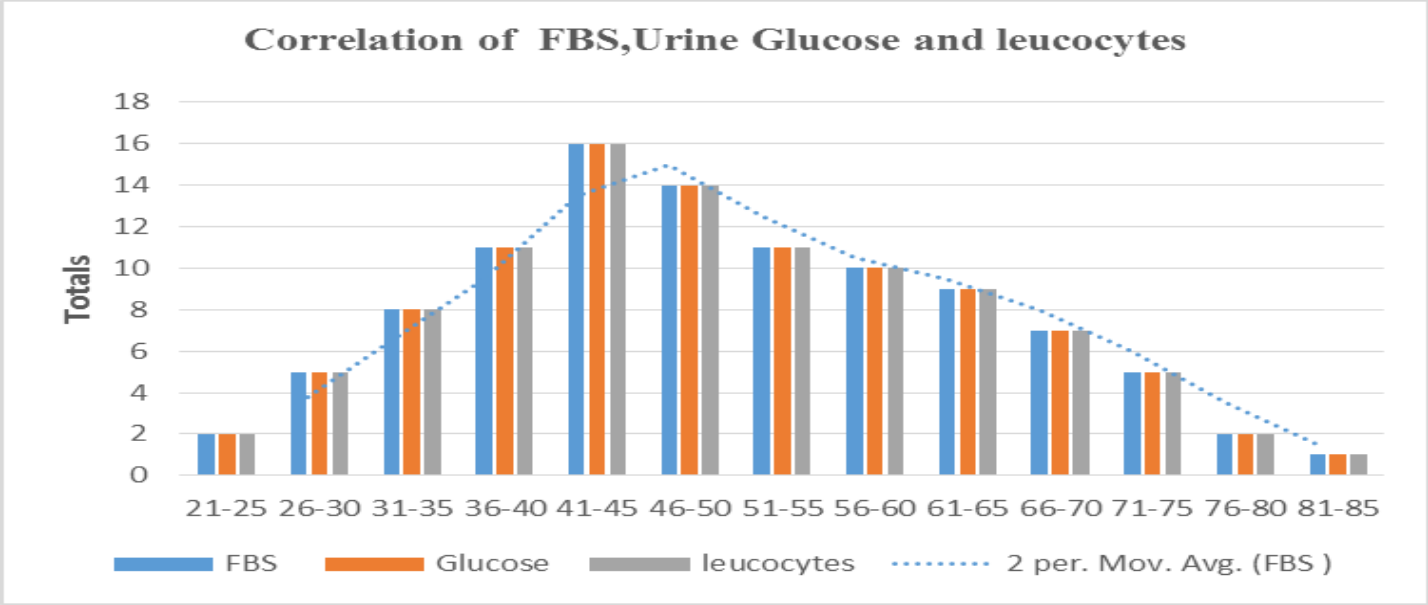

Figure 2. Correlation of Fasting Blood Sugar with Urine glucose and leucocytes
KEY; FBS= Values above 8mml/l for a known diabetic patient
Total Numbers indicates positivity patients' rate despite the intensity of the findings.

Table 2; Leaves extracts Inhibition Zones (mm)

Solvent	Concentration(mg/ml)	T1(mm)	T2(mm)	T3(mm)	Mean (mm)
Aqueous	800	9	9	9	9
	400	8	8.5	8	8.1
	200	8	8	8	8
	100	7	7	7	7
	Negative Control	-	-	-	-
	Positive Control (Ciprofloxacin25mg/mm)	34	34	34	34
Acetone	800	8.5	8	8	8.1
	400	8	7.5	7	7.5
	200	7.5	7	7	7.1
	100	7	6.5	6	6.5
	Negative Control	-	-	-	-
	Positive Control (Ciprofloxacin 25mg/mm	34	34	34	34
Ethyl acetate	800	9	9	9	9
	400	8	8	8	8
	200	7	7	7	7

	100	7	7	7	7
	Negative Control	-	-	-	-
	Positive Control (Ciprofloxacin 25mg/mm	34	34	34	34
Methanol	800	8	9	8	8.3
	400	7	8.5	7	7.5
	200	7	8	7	7.3
	100	7	7.5	6	6.8
	Negative Control	-	-	-	-
	Positive Control (Ciprofloxacin 25mg/mm)	34	34	34	34

Table 3: Stem barks inhibition zones (mm)

Solvent	**Concentration(mg/ml)**	**T1(mm)**	**T2(mm)**	**T3(mm)**	**Mean (mm)**
Aqueous	800	8	9	9	8.7
	400	7	8	8	7.7
	200	6	8	8	7.3
	100	5(no activity)	5	5	5
	Negative Control	-	-	-	-
	Positive Control (Ciprofloxacin 25mg/mm)	34	34	34	34
Acetone	800	8	8	8	8
	400	7	7	7	7
	200	7	7	7	7
	100	5	5	5	5
	Negative Control	-	-	-	-
	Positive Control (Ciprofloxacin 25mg/mm)	34	34	34	34
Ethyl Acetate	800	5	5	5	5
	400	5	5	5	5
	200	5	5	5	5
	100	5	5	5	5
	Negative Control	-	-	-	-
	Positive Control (Ciprofloxacin 25mg/mm)	34	34	34	34
Methanol	800	8	5	5	6
	400	8	5	5	6
	200	5	5	5	5
	100	5	5	5	5
	Negative Control	-	-	-	-

	Positive Control (Ciprofloxacin 25mg/mm)	34	34	34	34

Table 4: Leaves extracts MIC

a) Leaves extracts MIC 50

LEAVES EXTRACTS	**A. MIC 50**	**Serial dilutions mg/ml**	**Solvents**			
			Aqueous	**Acetone**	**Ethyl Acetate**	**Methanol**
		400	-ve	-ve	-ve	-ve
		200	-ve	-ve	-ve	-ve
		100	-ve	-ve	-ve	-ve
		50	-ve	-ve	-ve	-ve
		25	-ve	-ve	-ve	-ve
		Negative Control	Growth			
		Positive control	No growth			

b) Leaves extracts MIC 90

LEAVES EXTRACTS	**B. MIC 90**	**Serial dilutions mg/ml**	**Solvents**			
			Aqueous	**Acetone**	**Ethyl Acetate**	**Methanol**
		400	-ve	-ve	-ve	-ve
		200	-ve	-ve	-ve	-ve
		100	-ve	-ve	-ve	-ve
		50	-ve	-ve	-ve	-ve
		25	-ve	-ve	-ve	-ve
		Negative Control	Growth			
		Positive control	No growth			

Table 5: Stem barks

a) Stem bark MIC 50

STEM BARK EXTRACTS	**C. MIC 50**	**Serial dilutions mg/ml**	**Solvents**			
			Aqueous	**Acetone**	**Ethyl Acetate**	**Methanol**
		400	-ve	-ve	-ve	-ve

		200	-ve	-ve	-ve	-ve
		100	-ve	-ve	-ve	-ve
		50	-ve	-ve	-ve	-ve
		25	-ve	-ve	-ve	-ve
		Negative Control	Growth			
		Positive control	No growth			

b) Stem bark MIC 90

STEM BARK EXTRACTS	**MIC 90**	**Serial dilutions mg/ml**	**Solvents**			
			Aqueous	**Acetone**	**Ethyl Acetate**	**Methanol**
		400	-ve	-ve	-ve	-ve
		200	-ve	-ve	-ve	-ve
		100	-ve	-ve	-ve	-ve
		50	-ve	-ve	-ve	-ve
		25	-ve	-ve	-ve	-ve
		Negative Control	Growth			
		Positive control	No growth			

4. Discussion

Leaves

Inhibition Zones:

Leaves Aqueous and Ethyl acetate solvents were the best giving a mean inhibition zone of 9.0mm at 800mg/ml respectively whereas acetone was the poorest with a mean inhibition zone of 6.5 mm at 100mg/ml as shown in table 2

Minimum Inhibition Concentration:

Minimum inhibition zone of all the solvents in concentrations of 400, 200, 100, 50, 25, 10.5, 5.145 and 2.57 mgs/ml were Negative (Growth obtained). See tables 4 and 5.

Minimum Bactericidal Concentration:

Minimum Bactericidal Concentration procedure was not done due to negative MIC results (growth obtained) in all the plant parts extracts.

Stem barks

Inhibition zones (mm):

Stem bark aqueous solvent proofed to be the best with a mean inhibition zone of 8.7 mm at 800mg/ml unlike the ethyl acetate which gave the lowest mean inhibition zone of 5.0 in all the concentrations (800,400,200 and 100 mg/ml) as shown in table 3.

Minimum Inhibition Concentration:

Minimum inhibition zone of all the solvents in concentrations of 400, 200, 100, 50, 25, 10.5, 5.145 and 2.57 mgs/ml were Negative (Growth obtained See tables 4 and 5.

Minimum Bactericidal Concentration:

Minimum Bactericidal Concentration procedure was not done due to negative MIC results (growth obtained) in all the plant parts extracts.

Conclusions

This study showed that *Jatropha curcas* leaves and stem bark extracts singly and as a concoction are bacterial static against *E. coli* in Urinary Tract Infections of diabetic females and a prevalence rate of 69% of *Escherichia coli* clinical isolates as the causative agent of Urinary tract infection in diabetic females of Kitui County locality, Kenya.

Recommendation

1. Failure to proceed to Minimum Bactericidal Concentration from Minimum Inhibition Concentration as it is shown in tables 4 and 5, there was a static indication of the plant extracts to *E. coli* organism and thus further additional studies is worth on the medicinal plant.
2. There is need of further immunological analysis of *Jatropha curcas* crude extract of diabetic female with UTI for a bacterial static indicator may be a sign of immunological booster to the diabetic females with UTI.
3. Follow up of this study using clinical isolates from different body parts is needed.
4 Evaluation of adverse reactions is paramount.

Abbreviations

MBC: Minimum Bactericidal Concentration,
MIC: Minimum Inhibition Concentration, MSU: Mid-Stream Urine,
REMA: Resazurin-Based Microplate Assay
UTI: Urinary Tract Infection**.**

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