SEYBOLD Report
ISSN: 1533 - 9211
DOI: 10.5281/zenodo.7221755

PREVALENCE OF UROPATHOGENS AND ANTIMICROBIAL RESISTANCE PATTERN OF ISOLATES FROM GENERAL POPULATION IN CHENNAI, INDIA.

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ABSTRACT

The focus of this research was to determine the distribution of pathogenic organisms recovered from outpatients in the Chennai region of India, as well as their antibiotic susceptibility in vitro. A total of 745 urine samples were evaluated, with 340 (45.63 percent) showing signs of substantial bacterial growth. Urinary Tract Infections were identified based on positive culture growth. The Kirby-Bauer disc diffusion method was used to determine bacterial species and antimicrobial resistance of bacterial isolates against various popular antibiotics. From 340 instances, about 7 distinct types of uropathogens were discovered. *E. coli*, 139 isolates (40.8 %); *Klebsiella* spp, 86 isolates (25.2 %) *Proteus* spp., 61 isolates (17.9 %); *Pseudomonas* spp., 24 isolates (7%) coagulate negative *Staphylococci* spp (CNS), 12 isolates (3.5%) *S. aureus*. 11 (3.2%) isolates; *Candida* spp *7* isolates (2.0%).High resistance to Ampicillin, Erythromycin, Tetracycline, Ceftriaxone. Imipenem was indeed the antibiotic with the highest activity against isolated pathogenic organisms and the lowest resistance rate.

Keywords: Urinary tract infections, antibiotic resistance, Imipenem, Amikacin, *E. coli*.

Background:

Urinary infections (UTIs) are the most commonly seen disorders by doctors in underdeveloped nations, with a global prevalence of at least 250 million cases every year **[1, 2].** UTIs relate to the presence of microbial pathogens in the urinary system, and they can be symptomatic or asymptomatic, depending on the infection site (bladder [cystitis], urethra [urethritis], kidney [pyelonephritis], or urine [bacteriuria]. "Uncomplicated" infections are those that occur in a normal genitourinary tract with no prior instrumentation, whereas "complicated" infections are those that occur in genitourinary tracts with functional irregularities, including instrumentation such as catheterization urethral catheters, and are

SEYBOLD Report
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oftenly asymptomatic **[3].** At the global level, symptomatic UTIs are expected to cause up to 7 million visits to outpatient clinics, 1 million trips to emergency rooms, and 100,000 hospitalizations per year **[4].**

UTIs can be caused by a wide variety of bacteria, although Escherichia coli and other Enterobacteriacae are the most common pathogens in the community, accounting for over 75% of all isolates. In individuals with serious urinary tract infections and those who have been admitted to the hospital. Gram-negative bacteria, such as Pseudomonas spp., are more resistant to antibiotics. The proportional incidence of infections changes depending on the patient's age, gender, and length of stay in the hospital **[5].** UTIs are typically treated empirically, with treatment based on information obtained from the urine bacteria' antibiotic resistance pattern. Unchecked antibiotic use, on the other hand, has contributed to the evolution of resistant bacterial illnesses **[6-9].**

Antibiotic resistance amongst urinary pathogens is on the rise all around the world. The fact that a bacteria resistant to one antibiotic is frequently far more stable and resistant to other antibiotics, which drastically reduces the chances of getting a second empirical effort right. The prevalence of resistance to the most regularly prescribed medications for the treatment of urinary tract infections (UTIs) vary substantially around the world. The results of the study and susceptibility profile could help to determine which empirical treatment is the most effective **[10].** As a result, medical care professionals and health planners should look at the epidemiology of UTIs (prevalence, health conditions, microbial isolates, and antibiotic sensitivity) in order to determine the most appropriate interventions. As a result, the goal of this research was to determine the bacterial causative agent of pathogenic organisms and their in vitro sensitivity pattern to routinely used antimicrobial drugs. Uropathogens and their antimicrobial properties in male and females of various ages are highly effective.

MATERIALS AND METHODS

Study design

The study's goal was to figure out how uropathogens spread among UTI patients, as well as their in vitro susceptibility to various antimicrobials. In this study, 745 patients aged 18 to 40 years old who were suspected of having a urinary tract infection were assessed in outpatient settings in Chennai, Tamil Nadu, India. Out of 745 urine samples were collected and investigated, 340 of them were positive for significant bacterial growth. A medical Impact of these actions was developed for this investigation, which included the patient's sex, age, antibiotic resistance, and complaints. The suspicious patients' samples were examined and analysed for antimicrobial susceptibility using Kirby – Bauer disc diffusion procedures **[11]** (Bauer et al., 1996). The ethical committee has to provide their consent.

Urine sample collection and determination of infection

Midstream urine samples were collected aseptically from the UTI patients with sterile screw-lid labelled urine containers (100 ml) followed by Thomson and Miller **[12]**(Thomson, 2003).10µl of urine samples were used and plated for quantitative culture on nutrient agar media prepared according to the established methods. 10^5 colony forming unit (CFU) /ml was

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considered as positive culture **[13]** (Cheesbrough, 2006).

Isolation and Identification of Uropathogens

Pathogens were isolated from the urine sediment. 10 µl of urine samples were cultured on the Blood Agar and MacConkey Agar by using a standard calibrated loop (0.01ml) and the plates were incubated at 35°C for 24 hrs.

Identifications of uropathogens

Colony morphology, size, shape, elevation, pigmentation were observed and gram staining was performed.

Biochemical Examination

The identified colonies were chosen based on cultural, microscopic, and microbiological studies, and then biochemical tests (indole, urease, TSI, citrate, mannitol, motility, oxidase and catalase) were performed to confirm the presence of pathogenic organisms.

Antibiotic susceptibility test

The Kirby – Bauer disc - diffusion technique was used to perform an antibiotic susceptibility test according to the Clinical and Laboratory Standards Institute (CLSI) guidelines **[14]** (CLSI, 2013). ref J. Budd, A. Durham, T. Gwise, and others The FDA has issued a guideline for comparing measurement processes and evaluating bias using patient samples. Clinical and Laboratory Standards Institute, Wayne (PA), document EP09-A3, 2013. According to the manufacturer's recommendation the Muller Hinton agar media (38g/L) was prepared and antibiotic susceptibility test was performed **[11]** (Bauer et al., 1966). The suspected isolates were grown in peptone water and incubated for 8 hours at 37°C and compared to 0.5Mc Farland standard. The grown bacterial culture was lawn cultured on Muller – Hinton agar plates and commercially available antibiotic discs (30µg) (Amikacin (AIK), Nitrofurantoin (NIT),Ceftriaxone (CTR),Ciprofloxacin (CIP), Imipenem (IPM), Gentamicin (GEN), Ofloxacin (OF), Amoxicillin/clavulanic acid (AMC), Ampicillin (AMP), Tetracycline (TE)Erythromycin (ER), Doxycycline hydrochloride (DO), Chloramphenicol , Co Trimoxazole (COT) were placed on the medium by using sterile forceps and to ensure the working condition of the disc, ATCC strains of each microbes were also included and the plates were incubated at 37°C for 24 hrs. The diameter of zone of inhibition was measured and the results were interpreted according to CLSI standard interpretative charts.

RESULTS

Demographic and clinical characteristics of patients

A number of 745 urine samples were evaluated, with 340 (45.63 percent) revealing substantial bacterial growth (105 CFU/mL). Male and female patients aged 20 to 50 years old had uropathogenic bacterial species isolated. The female age group of 21 to 30 years old had a significant prevalence of UTI (55 percent), which was higher than the other age groups. (See Table 1) Table 2 summarizes the prevalence and gender distribution of UTI cases.

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Table 1. Distribution of uropathogenic isolates according to the age groups and Male and Female

Age groups (years) distribution	No. (%) of bacteria	Male (No of Growth)	Female (No of Growth)	Total (No of Growth)
<20	39 (11.47 %)	13	26	39
21–30	189 (55.58 %)	71	118	189
31–40	88 (25.88 %)	32	56	88
41–50	24 (7.05 %)	9	15	24
Total No. (%)	340 (100 %)	125	215	340

Identification of uropathogens

The most common Gram negative uropathogens were isolated viz., *E.coli*, 139 isolates (40.8%); *Klebsiella* spp, 86 isolates (25.2 %) *Proteus* spp., 61 isolates (17.9 %); Pseudomonas spp., 24 isolates (7%); coagulate negative *staphylococci* (CNS), 12 isolates (3.5%) *S. aureus*, 11 (3.2%) isolates (Fig. 1).

Table 2. Frequency of gender distribution of UTI cases

Demographic characteristics	PositiveNo. (%)	Negative No. (%)	Total (%)
Male	78 (33.1)	157 (66.8)	235 (100)
Female	262 (51.3)	248 (48.6)	510(100)
Total	340(45.6)	405 (54.3)	745 (100)

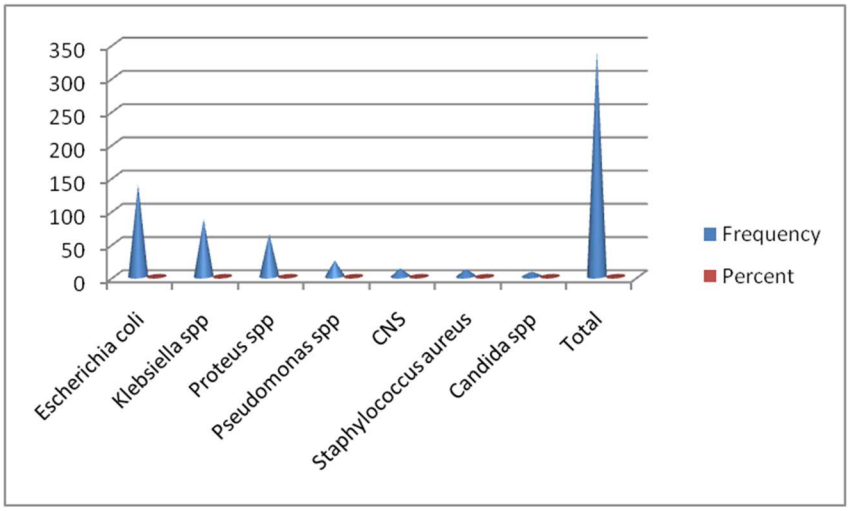

Fig.1 Identification of uropathogens

The Kirby Bauer Disc Diffusion technique was used to test the antimicrobial sensitivity of all the microorganisms. The size of the inhibition zones around each disc was measured with Vernier callipers on the back of the plate, with reflected light against a dark non-reflected

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ISSN: 1533 - 9211
DOI: 10.5281/zenodo.7221755

background, at the end of the incubation period. 14 commonly used antibiotics were tested against the isolated bacteria. Imipenem was determined to be the most effective drug, followed by Amikacin and Gentamicin (GEN) and Nitrofurantoin. Isolated urinary tract pathogens, including *E. coli, Klebsiella spp., Proteus spp., and Pseudomonas spp,Staphylococcus spp*, and *Staphylococcus aureus* (Fig 2).

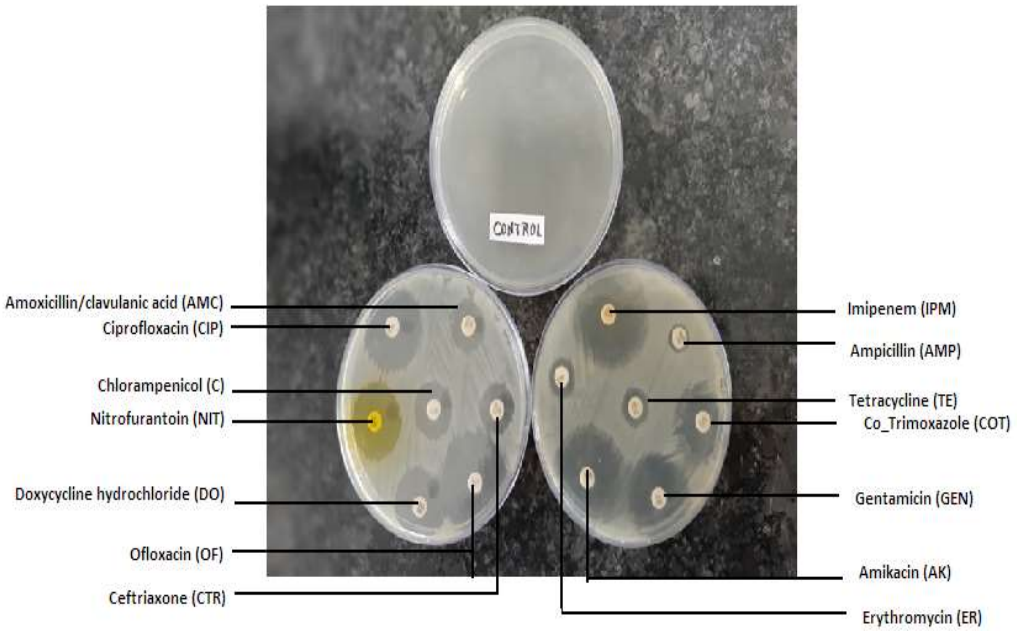

Fig.2 Antibiotic sensitivity pattern of isolated uropathogens

The discrepancies in the percentage of uropathogens could be attributed to changes in community customs, environmental factors, personnel cleanliness procedures, and health-care facilities. Proteus spp., and Pseudomonas spp., was the other uropathogenic bacteria found in this investigation; this finding is consistent with other studies that have shown the isolation of these bacteria in low percentages when compared to *E.coli* and *Klebsiella spp*. These bacteria are also regarded as human opportunistic pathogens and causative agents of nosocomial illnesses. The antibiotic resistance pattern of uropathogenic isolates is shown in Table 3.

Table 3 Antibiotic resistance of the uropathogenic bacteria isolated from Patients with suspected UTI

Antibiotic	***E.coli***	***Klebsiella spp***	***Proteus spp***	***Pseudomonas spp***	***CNS***	***S.aureus***
Drugs	**No of R-Test**	**No of R-Test**	**No of R-Test**	**No of R-Test**	**No of R-Test**	**No of R-Test**
CTR	69	43	34	23	5	5
IPM	2	2	3	1	1	1
AK	28	25	11	3	2	2

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OF	48	38	13	10	4	3
GEN	35	30	27	9	2	2
CIP	45	28	25	6	3	2
NIT	43	19	60	6	3	3
AMP	86	85	56	23	8	6
AMC	50	38	28	23	3	4
DO	44	35	18	8	3	2
C	41	32	19	9	2	2
COT	50	32	20	9	3	3
TE	77	55	60	23	6	7
E	73	54	39	12	6	8

R=Resistance

The final findings of resistant bacteria of isolated bacteria revealed the following proportion of resistance: Intrinsic resistance medicines cause high resistance to ampicillin, tetracycline, ceftriaxone, and erythromycin. The heavy active antibiotics against isolated uropathogens with the lowest resistance prevalence were imipenem and amikacin. (Fig. 3).

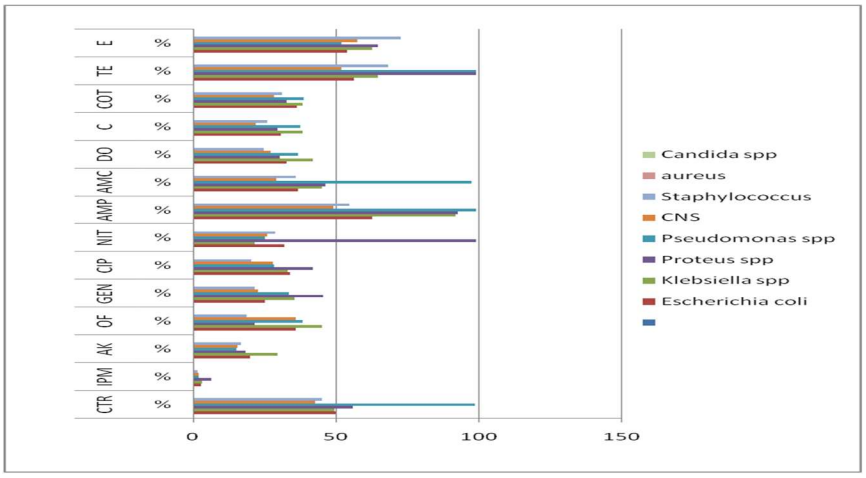

Fig. 3.Antimicrobial resistance of isolated uropathogens

DISCUSSION

UTIs are among the most often diagnosed disorders in the world. The availability of novel antimicrobials has improved the treatment of urinary tract infections. However, the advent of antimicrobial treatment resistance has put the process of UTI illnesses in jeopardy.

Overall, 22.7 percent of uropathogens were isolated in this investigation, which is lower than reported rates from Ethiopia and Nigeria [**15].** However, the rate was higher than in previous studies **[16]**. Gram negative bacteria were found to be more important for UTI than Gram - positive organisms, which is consistent with prior research **[17, 18].** E.coli and Klebsiella spp., Proteus spp., Pseudomonas spp., CNS, S.aureus, Enterobacter spp., and Citrobacter spp. were the most common bacteria identified from urine. The frequencies of E.

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coli and other pathogens isolates in this investigation were comparable to those previously reported **[19].** The rates, on the other hand, were often lower than in earlier reports. **[20, 21].**

The relative abundance of bacteria is known to be influenced by differences in identification procedures, making comparison difficult **[22].** Bacterial etiologies of urinary tract infections might vary geographically and even over time within a population **[23].**

The majority of pathogens were recovered from females (P 0.001), indicating a statistically significant difference between genders. Females and males have different prevalence rates, according to studies conducted all around the world **[24].** Male and female differences are explained by physiological and anatomical differences.

This is because, in comparison to females, the urethra's dry environment hinders germs from growing to their full potential. Prostate secretions' antibacterial action and the longer distance between both the anus and the urethra are two factors that contribute to the disparity in prevalence between men and women**[25].** The female urethra and vagina have an anatomical link that makes them vulnerable to injuries during sexual contact as well as microorganisms being rubbed up the urethral into the bladder throughout pregnancy and childbirth **[26].** The prevalence of uropathogens among age categories was statistically significant (P=0.011), with uropathogens being more prevalent in fertile age groups than others. This outcome was in line with the results of a study conducted in India **[27].**

The most common bacterial isolates were found to be resistant to erythromycin, amoxycillin, and tetracycline but responsive to nitrofurantoin. This is comparable to what has been observed in Ethiopia and Nigeria **[28].** Amoxycillin, erythromycin, and tetracycline resistance was discovered in Klebsiella spp., Proteus spp., and Pseudomonas spp., although gentamicin and ciprofloxacin sensitivity was discovered in *Klebsiella spp., Proteus spp., and Pseudomonas spp.* Amoxycillin, erythromycin, and tetracycline showed statistically significant resistance rates (P0.001). These figures are higher than those in Ethiopia and other nations. Previous studies **[29]** have shown that antibiotic resistance to these and many other antimicrobials is increasing.

E. coli was discovered to be resistant to nitrofurantoin. Other isolates were resistant to gentamicin and ciprofloxacin. Previous studies have shown high rates of susceptibility to nitrofurantoin **[30],** ciprofloxacin, and gentamicin **[31].** Resistance to two or more antimicrobial drugs was found to be 74.9 percent in this study. A similar finding was found in a prior investigation in Ethiopia **[32].**

The results of routine microbiological testing conducted in 2018 and 2019 were used in this retrospective analysis. We were unable to track patients' clinical settings due to the retrospective character of the study. As a result, characteristics such as outpatient, catheterized and non-catheterized patients were not considered in the study.

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The majority of UTIs were documented in this study from females, showing that women are more likely than men to have UTI. Our study found that age groups 21–30 and 31–40 were more affected by the illness than other age groups, most likely due to anatomical and physiological changes.

The study has established that for *E.coli* infected UTI patient's suitable treatment was amikacin, imepenem. Low resistance was found to amikacin and Imepenem despite the high level of resistance to the other drugs like Ampicillin, Erythromycin, Tetracycline, and ceftriaxone. Impenem, Nitrofurantoin and Amikacin were the more active antibiotic in *Klebsiella spp. Proteus spp*: Intrinsic resistance antibiotic drug was Ampicillin, Tetracycline, Nitrofurantoin, and Amoxicillin/clavulanic acid. Imipenem, Amikacin and Ofloxacin were the most active antibiotic in uropathogens. *Pseudomonas* spp infected patients to right treatment was Imipenem, Amikacin and Nitrofurantoin. Resistance antibiotic drug was Ampicillin, Tetracycline, Ceftriaxone, and Amoxicillin/clavulanic acid. *CNS satisfactory* drugs were Imipenem and Amikacin. Ampicillin, Erythromycin, Tetracycline, Ceftriaxone, and Ofloxacin (OF) was highly resistance. *Staphylococcus aureus*: High resistance to Ampicillin, Erythromycin Tetracycline, Ceftriaxone, Amoxicillin/clavulanic acid. Imipenem and Amikacinwas the more active antibiotic in UTI patients.

The bacterium that was most commonly isolated was resistant to Amikacin and Ofloxacin, whereas the other isolates were resistant to Nitrofurantoin. The antibiotic imipenem was more effective against uropathogens. Antimicrobials such as Nitrofurantoin, Gentamicin, Ciprofloxacin, and Ofloxacin are regarded acceptable for empirical therapy of UTI in the area. It is advised that the aetiology and drug susceptibility be monitored on a regular basis.

Conflict of Interest: None

REFERENCES:

1. Ronald AR, *et al*. *Int J Antimicrob Agents.* 2001 **17**:343-348.

2. Barisˇic´ Z, *et al. Intl J Antimicrob Agents.* 2003 **22**: S61-S64.

3. Gonzalez CM, Schaeffer AJ. *World J Urol.* 1999 **17**:372–382.

4. Wilson ML, Gaido L. *Clin Infect Dis.* 2004 **38**:1150–1158.

5. Sefton AM. *Int J Antimicrob Agents.* 2000 **16**:489–491.

6. Bonadio M, et al. *Eur J Urol* 2001 **40**:439-445.

7. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disc susceptibility tests. 7th ed. Wayne, Pennsylvania, USA: NCCLS; **2000**. M2-A7.

8. Grude N, Tveten Y and Kristiansen BE. *Clin Microbiol Infect.* 2001 **7**:543—547.

DOI: 10.5281/zenodo.7221755

9. Kripke C. *Am Fam Physician* 2005 **72**:2219.

11. Sundqvist M, Kahlmeter G. *J Antimicrob Chemother.* 2009 **64**:227–228.

10. Farajnia S, et al. *Int J Infect Dis.* 2009 **13**:140—144.

11. Bauer BE, et al. *Mol Biol Cell.* 1996 **7(10):**1521-33

12.Thomson, 2003

13.Cheesbrough, 2006

14.CLSI, 2013

15. Biadglegne F, Abera B. *Ethiop J Health Dev*. 2009 **23**:236–238.
16. Kashef N, Djavid GE and Shahbazi S. *J Infect DevCtries*. 2010 **4**:202–206.
17. Theodros G. *Revista CENIC. CienciasBiológicas.* 2010 **41**:1–6.
18. Beyene G, Tsegaye W. *Ethiop J Health Sci.* 2011 **21**:141–146.
19. Demile T, et al. *Ethiop J Health Sci.* 2012 **22**:121–128.
20. Bahadin J, Teo SSH and Mathew S. *Singapore Med J.* 2011 **52**:415–420.
21. Tseng MH, et al. *Pediatr Int.* 2008 **50**:797–800.
22. Leegaard TM, et al. *Clin Microbiol Infect*. 2000 **6**:290.
23.El-Mahmood et al. *J Clin Med Res.* 2009 **1**:26–34.
24. Kibret M, Abera B. *Asian Pac J Trop Biomed.* 2014 **4(2):**164-168.
25. Hooton TM. *J AntimicrobChemother*. 2000 **46**(Suppl 1):1–17.
26. Kolawale AS, et al. *Int J Med Sci.* 2009 **1**:163–167.
27. Desai P, et al. *Int J Biol Med Res.* 2012 **3**:2007–2012.
28. Melaku S, et al. *Afr Health Sci.* 2012 **12**:134–139.
29. Khoshbakht R, et al. *J Micribiol.* 2013 **6**:86–90.
30. Iregbu KC, et al. *Afr J ClinExpMicrobiol*.2013 **14**:169–173.
31. Niladri DS, Kuhu P. *J Drug DelivTher*.2013 **3**:16–19.
32. Yismaw G, et al. *Eur J Exp Biol.* 2012 **2**:889–898.