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# Phylogenetic analysis of multidrug resistant *E. coli* isolates from the urinary tract in Bushenyi district, Uganda using the new Clermont phylotyping method

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**Due to the increasing rates of multidrug resistance (MDR) among the Enterobacteriaceae that cause urinary tract infections (UTIs), selection of antimicrobial agents for empirical therapy is becoming a major challenge. This study determined the antimicrobial resistance profiles, multidrug resistance profiles, multiple antibiotic resistance indices (MARI), factors associated with MDR UTIs and the phylogenetic groups of MDR *Escherichia coli* strains isolated from the urinary tract among patients attending hospitals in Bushenyi District, Uganda. In this cross-sectional study, a total of 86 bacterial uropathogens isolated from 267 study participants suspected to have UTIs were subjected to antimicrobial susceptibility tests using the Kirby Bauer Disk diffusion method. Data for the factors associated with MDR were obtained by the use of questionnaires. Phylogenetic groups of the MDR *E. coli* were determined using the new Clermont method for phylotyping *E. coli*. Descriptive and multiple logistic regression statistical tools were used to determine phylogenetic groups, and assess for statistically significant relationship between MDR UTIs and factors suspected to be associated with MDR UTIs respectively. The isolates assigned as group B2 9/12 (75.0%), B1 2/5 (40.0%) and A 2/7 (28.6%) by using the old Clermont method could not be phylotyped using the new Clermont method and were grouped as non-typeable strains of *E. coli*. Our study demonstrated high prevalence of the non-typeable strains of MDR *E. coli*, we therefore recommend the use of modern DNA sequencing-based approaches which is the gold standard for genotyping bacteria, that this current study could not afford.**

**Key words:** Phylogenetic analysis, bacterial urinary tract infections, factors associated with, multidrug resistance, Bushenyi District, Uganda.

## INTRODUCTION

Urinary tract infections are one of the most common infectious diseases, which damages different parts of the urinary system such as urethra, bladder, ureters and the kidney (Anwar et al., 2018). It is estimated that about 150 million people per year are diagnosed with UTIs costing greater than 6 billion US dollars used for health care (Stamm and Norrby, 2001; Gupta et al., 2001; Laupland et al., 2007; Ali et al., 2017). Urinary tract infections are commonly treated with β-lactam antibiotics and fluoroquinolones but the emergence of MDR strains among the leading uropathogens to commonly used antimicrobial agents is on the rise, resulting into impaired treatment of UTIs (Shabbir et al., 2018). According to Khawcharoenporn et al. (2013) and Hadifar et al. (2016), MDR was described as the non-susceptibility of bacteria to at least one antimicrobial agent in 3 or more antimicrobial classes. The development of the MDR strains in bacterial uropathogens has been attributed to several factors such as female gender, older age, history of UTIs, residence in the nursing home, hospitalization, prior exposure to antimicrobials, urinary catheterization and bacterial factors (Shabbir et al., 2018; Hasan et al., 2007).

Clermont et al. (2000) coined a triplex polymerase chain reaction (PCR) amplification technique based on the *chuA, yjA* and Tspe4.C2 genes' analysis to categorized *E. coli* into the following phylogenetic groups; A, B1, B2, or D (Clermont et al., 2000). Multilocus sequence type (MLST) data clearly indicated that 80-85% of the phylogenetic group allocations where actually correct (Iranpour et al., 2015) while a smaller fraction of *E. coli* strains with A0, D1 and D2 genotypes were not assigned into correct phylo-groups. Clermont et al. (2013) added *arpA* gene to the triplex PCR to make a quadruplex PCR. The addition of this new marker, *arpA*, improved the specificity and detection of new phylogenetic groups in *E. coli* including; A, B1, B2, C, D, E, F, and clade I (Clermont et al., 2013).

The increase in the rise of drug resistant bacteria resulted into MDR strains (Li and Webster, 2018). In Uganda, the policy of treatment of UTIs was put in place but susceptibility patterns of these bacteria seem to be changing (Kabugo et al., 2016). Most of the previous studies in Uganda by Mwaka et al. (2011), Odongo et al. (2013), Odoki et al. (2015), Katongole et al. (2015), Ampaire et al. (2015) and Kabugo et al. (2016) focused much on the prevalence and antimicrobial susceptibility patterns of bacterial uropathogens neglecting MDR factors associated with these bacteria and their phylogenetic groups. Khawcharoenporn et al. (2013) reported that, Infectious Diseases Society of America recent guidelines recommended that treatment of UTIs using antimicrobial therapy should be directly proportional to indigenous resistance profile of bacteria, drug availability and antimicrobial intolerance/allergy history of treated patients (Gupta et al., 2011). Due to that, indigenous epidemiological studies are vital in the choice of the most proper antimicrobials for empirical treatment, so as to combat the spread of MDR bacterial uropathogens within our communities and healthcare centers. To date there is no study focusing on the MDR bacterial uropathogens, factors associated with MDR UTIs and phylogenetic groups of the bacteria causing MDR UTIs in Bushenyi District, Uganda. Therefore, this study was designed to determine antimicrobial resistance profiles, multidrug resistance profiles, MARI, factors associated with MDR UTIs, and the phylogenetic groups of MDR *E. coli* strains isolated from the urinary tract among patients attending hospitals in Bushenyi District, Uganda.

## MATERIALS AND METHODS

### Study design

This was a cross-sectional health-point survey conducted from June, 2017 to January, 2018 on 86 bacterial uropathogens isolated previously from 267 study participants suspected to have UTIs that attended Kampala International University-Teaching Hospital (KIU-TH), Ishaka Adventist Hospital and Comboni Hospital Kyamuhunga by Odoki et al. (2019). This study used a survey formula previously reported by Kish (1965):

$$n = z^2 p\,(1-p)/d^2$$

Where d=margin of error of setting a significance level of 0.05 (5%); z=level of significance (1.96) for confidence interval of 95%; p = prevalence of UTIs in Bushenyi District of Uganda, among patients receiving medical treatment at selected hospitals was 22.33% (Tibyangye et al., 2015). Patients of the following category were included in the study: symptomatic UTIs, suspected to have UTIs, and residents of Bushenyi District that are receiving medical treatment at KIU-TH, Comboni Hospital kyamuhunga and Ishaka Adventist Hospital. Patients of the following category were excluded from the study: critically ill, menstruating females, those unable to micturate and those who are currently on antibiotics or with history of antibiotic usage in a fortnight. Patients who met the selection criteria from each of the respective hospitals were recruited in the study using simple random sampling technique (Odoki et al., 2019).

### Study variables

Questionnaires were administered to collect information from the study participants as regards sociodemographic data such as: age, gender, residence, marital status, level of education, circumcision and sexual intercourse. Data on the health status were obtained by the clinicians through clinical examinations and medical history of the study participants like: hypertension, genitourinary abnormalities, abortion, recurrent UTIs, previous hospital admission,

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family history of UTIs, previous UTIs, chronic respiratory disease, wrong prescription of antimicrobial agents, history of fluoroquinolone, cephalosporin and any antimicrobial use in the last 12 months. Data on selected factors suspected to be associated with MDR UTIs such as pregnancy, diabetes mellitus and human immunodeficiency virus (HIV), were obtained through laboratory investigations (Odoki et al., 2019).

### Antimicrobial susceptibility testing

The antimicrobial susceptibility tests were done at Mbarara University of Science and Technology-Teaching Hospital (MUST-TH) microbiology laboratory on bacterial isolates previously isolated and characterized by Odoki et al. (2019). Antimicrobial susceptibility tests were performed on bacterial isolates from midstream urine (MSU) using antimicrobial discs, according to Clinical and Laboratory Standards Institute (CLSI) on Muller Hinton agar (CLSI, 2018). The prepared media was inoculated with bacterial suspension equivalent to 0.5 MacFarland turbidity. The commercially available antimicrobial discs containing the following antimicrobials: ampicillin (10 µg), nitrofurantoin (50 µg), cefoxitin (30 µg), co-trimoxazole (25 (1.25/23.75) µg), amikacin (30 µg), gentamicin (10 µg), imipenem (10 µg), and erythromycin (15 µg) (Himedia, India) were aseptically placed on the surfaces of the sensitivity agar plates with a sterile forceps and allowed to stand for 30 min. The plates were then incubated for 18-24 h at 37°C. Zones of inhibition after incubation were observed, measured and interpreted according to CLSI (2018). For each antimicrobial agent used, results were reported as sensitive or resistant. Isolates showing intermediate antimicrobial susceptibility were considered to be resistant. *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as quality control organisms for the antimicrobial susceptibility testing according to CLSI (2018). Multidrug resistant isolates were defined as isolates that demonstrated resistance to ≥ 3 of the following antimicrobial agent categories as previously reported by Khawcharoenporn et al. (2013) and Hadifar et al. (2016): (1) penicillin: ampicillin, (2) nitrofurans: nitrofurantoin, (3) cephalosporins: cefoxitin, (4) trimethoprime-sulphonamides: co-trimoxazole, (5) aminoglycosides: gentamicin and amikacin, (6) carbapenems: imipenem and, (7) macrolides: erythromycin.

### Multiple antibiotic resistance indices

Calculation of MARI was done by dividing the number of antimicrobial agents that the bacterial uropathogen is resistant to, by the total number of antimicrobial agents to which the bacterial uropathogen was tested against (Ekwealor et al., 2016). Multiple antibiotic resistance index of ≥0.2 is an indication that this particular bacterial colony can be traced back to a habitat where several antimicrobial agents have been used or abused (Ehinmidu, 2003; Tambekar et al., 2006; Oli et al., 2013).

### Phylogenetic analysis of the MDR *E. coli* isolates

The molecular biology tests to determine the phylogenetic groups of MDR *E. coli* strains using the new Clermont phylotyping method were done at Makerere University, College of Health Sciences, Molecular Biology Laboratory, Mulago, Kampala, Uganda. Deoxyribonucleic acids were extracted from pure colony of 31 different isolates of MDR *E. coli* by boiling method as previous described by Acaku et al. (2014). Simplex PCR for genotyping was done using primer flacking genomic regions in *chuA, yjaA,* TsPE4.C2 and *arpA* with slight modifications as previously described by Sáez-López et al. (2016). Polymerase chain reactions were done in a 20 µL Volume containing 2Xtaq master (NEB, USA) 10 pmol of forward and reverse primer as well as nuclease free water using these PCR conditions. Initial denaturation at 95°C for 5 min, denaturation at 94°C for 30 s, annealing at 52°C for 30 s for 30 cycles, extension at 6°C for 30 s for 30 cycles and final extension at 68°C for 10 min. Following amplification, the amplicons were analyzed by agarose electrophoresis using a 2% TBE- Agarose gel containing ethidium bromide. The gels were viewed and UVP gel imaging system-Bio DOC-It (CA, USA) to analyze an expected amplicon size (Table 1). Deoxyribonucleic acids were extracted from the control organism and were used as positive controls while PCR water was used for negative extraction as well as negative template controls. The *trpA* gene was used as an internal control on all isolates and several *E. coli* strains previously characterized and known to harbor; *chu*A*, yja*A*, arp*A*, trp*A genes and TspE4.C2 at Makerere University, College of Health Sciences, Molecular Biology Laboratory, Mulago, Kampala, Uganda were used as positive controls. The gene amplification results were considered to be positive, if the PCR products were similar to the expected amplicon size.

### Data analysis

Data involving patients' socio-demographic characteristics, health conditions, MDR profiles and phylogenetic groups of MDR *E. coli* strains were entered in Microsoft Excel and exported to IBM SPSSv20 software for analysis. The MDR profiles and distribution of MDR among the bacterial uropathogens was determined using descriptive statistics. Descriptive statistics was also used to obtain the prevalence of phylogenetic groups of the MDR strains of *E. coli* according to the old and new Clermont phylotyping methods. The result of MDR UTIs was categorized into: presence or absence of MDR. Bivariate analysis was used to determine whether statistically significant relationship exists between MDR UTIs and factors suspected to be associated with MDR UTIs. Stepwise forward multiple logistic regression model was applied on all variables with *p* value ≤0.2 to assess the statistically significant relationship (p≤0.05) to eliminate confounding variables.

### Ethical approval

Ethical clearance was obtained from MUST, Institutional Research and Ethics Committee (IREC) on Human Research (No. 01/01-17) and final clearance was sought from Uganda National Council for Science and Technology (UNCST) (No. HS 2232). The Helsinki declaration of 1964 revised in 2000 ethical principles was observed on all research protocols (WMADH, 2000).

## RESULTS

### Multidrug resistance profiles of the bacterial uropathogens

When the bacterial uropathogens were subjected to the following seven different categories of antibiotics; (1) penicillin: ampicillin, (2) nitrofurans: nitrofurantoin, (3) cephalosporins: cefoxitin, (4) trimethoprime-sulphonamides: co-trimoxazole, (5) aminoglycosides : gentamicin and amikacin, (6) carbapenems: imipenem and, (7) macrolides: erythromycin, the most resisted antimicrobial agents were erythromycin with 74/86 (86.0%) and co-trimoxazole 74/86 (86.0%), followed by ampicillin 69/86 (80.2%), and cefoxitin 37/83 (44.6%) (Table 2).

**Table 1.** Primer sequences, sizes of PCR products used in phylotyping MDR strains of *E.coli* using a simplex PCR method.

| PCR reaction | Primer ID | Target | Primer sequences | PCR product (bp) | References |
|---|---|---|---|---|---|
| Simplex | chuA.1b<br>chuA.2 | *chuA* | 5′-ATGGTACCGGACGAACCAAC-3′<br>5′-TGCCGCCAGTACCAAAGACA-3′ | 288 | Clermont et al. (2000) |
| Simplex | yjaA.1b<br>yjaA.2b | *yjaA* | 5′-CAAACGTGAAGTGTCAGGAG-3′<br>5′-AATGCGTTCCTCAACCTGTG-3 | 211 | Clermont et al. (2000) |
| Simplex | TspE4C2.1b<br>TspE4C2.2b | TspE4.C2 | 5′-CACTATTCGTAAGGTCATCC-3′<br>5′-AGTTTATCGCTGCGGGTCGC-3′ | 152 | Clermont et al. (2000) |
| Simplex | AceK.f<br>ArpA1.r | *arpA* | 5′-AACGCTATTCGCCAGCTTGC-3′<br>5-TCTCCCCATACCGTACGCTA-3 | 400 | Clermont et al. (2004) |
| Group E | ArpAgpE.f<br>ArpAgpE.r | *arpA* | 5′-GATTCCATCTTGTCAAAATATGCC-3′<br>5-GAAAAGAAAAAGAATTCCCAAGAG-3 | 301 | Lescat et al. (2012) |
| Internal control | trpBA.f<br>trpBA.r | *trpA* | 5′-CGGCGATAAAGACATCTTCAC-3′<br>5′-GCAACGCGGCCTGGCGGAAG-3′ | 489 | Clermont et al. (2008) |

**Table 2.** Antibiotic resistant profiles of the bacterial uropathogens.

| Uropathogens | AMP | NI | CX | SXT | AK | GEN | IMI | E |
|---|---|---|---|---|---|---|---|---|
| *E. coli n*=36 | 29 (80.6) | 5 (13.9) | 13 (36.1) | 35 (97.2) | 0 (0.0) | 3 (8.3) | 3 (8.3) | 36 (100.0) |
| *K. pneumoniae*=10 | 7 (70.0) | 5 (50.0) | 1 (10.0) | 10 (100.0) | 0 (0.0) | 1 (10.0) | 4 (40.0) | 10 (100.0) |
| *K. oxytoca*=6 | 6 (100.0) | 2 (33.3) | 1 (16.7) | 4 (66.7) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 6(100.0) |
| *P. mirabilis*=3 | 3 (100.0) | 2 (66.7) | 3 (100.0) | 3 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 3 (100.0) |
| *P. vulgaris*=1 | 0 (0.0) | 0 (0.0) | 1 (100.0) | 1 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (100.0) |
| *S. aureus*=27 | 22 (81.5) | 2 (7.4) | 18 (66.7) | 19 (70.4) | 9 (33.3) | 10 (37.0) | 2 (7.4) | 15 (55.6) |
| *E. faecalis*=3 | 2 (66.7) | 1 (33.3) | -- | 2 (66.7) | -- | -- | 0 (0.0) | 3 (100.0) |
| Total | 69 (80.2) | 17 (19.8) | 37 (44.6) | 74 (86.0) | 9 (10.8) | 14 (16.9) | 9 (10.5) | 74 (86.0) |

AMP=ampicillin; NI=nitrofurantoin; CX=cefoxitin; SXT=co-trimoxazole; AK=amikacin; GEN=gentamicin; IMI=imipenem; E=erythromycin; "--"= not done.

## Distribution of MDR and MARI

When the bacterial uropathogens were subjected to ampicillin, nitrofurantoin, cefoxitin, co-trimoxazole, gentamicin, amikacin, imipenem and erythromycin, selection of MDR strains were in the order: 6/6 (100.0%) *K. oxytoca*, 3/3 (100.0%) *P. mirabilis*, 1/1 (100.0%) *P. vulgaris* > 9/10 (90.0%) *K. pneumoniae* > 31/36 (86.1%) *E. coli* > 22/27 (81.5%) *S. aureus* > 1/3(33.3%) *E. faecalis* (Table 3). All the bacterial uropathogens tested showed MARI of ≥0.2 (Table 4).

**Table 3.** Distribution of MDR among the bacterial uropathogens.

| Uropathogens | MDR strains n (%) |
|---|---|
| *E. coli* n=36 | 31 (86.1) |
| *K. pneumoniae* n=10 | 9 (90.0) |
| *K. oxytoca* n=6 | 6 (100.0) |
| *P. mirabilis* n=3 | 3 (100.0) |
| *P. vulgaris* n=1 | 1 (100.0) |
| *S. aureus* n=27 | 22 (81.5) |
| *E. faecalis* n=3 | 1(33.3) |
| Total | 73 (84.9) |

n=number; %=percentage.

### Factors associated with MDR UTIs

When the predictor variables for MDR were subjected to bivariate analysis, they had the following logistic regression values: hospitalization (OR=3.616; 95% CI: 1.017-12.860; p<0.05) and previous use of any antimicrobial agent in the last 12 months (OR=5.175; 95% CI: 1.007-26.605; p<0.05) were found to be statistically significant (p<0.05) with MDR UTIs (Tables 5 and 6). When all the variables with a *p* value of 0.2 or less were entered into stepwise forward multiple logistic regression model, they had the following logistic regression values: hospitalization (OR=3.947; 95% CI: 1.050-14.831; p<0.05) and previous use of any antimicrobial agent (OR=6.004; 95% CI: 1.046-34.454; p<0.05) were found to have statistically significant relationships (p<0.05) with MDR UTIs (Table 7). However, age, gender, residence, marital status, level of education, sexual intercourse, pregnancy, hypertension, genitourinary tract abnormalities, diabetes mellitus, HIV, cephalosporin use in the last 12 months, fluoroquinolone use in the last 12 months, abortion, chronic respiratory disease, recurrent UTIs, previous hospital admission, family history of UTIs, previous UTIs and wrong prescription of antimicrobial agents were found to have no significant association with MDR UTIs (Tables 5 and 6).

### Determination of the phylogenetic groups of MDR *E. coli* strains

The results obtained in the phylotyping of the MDR *E. coli* strains isolated from urinary tract of patients attending hospitals in Bushenyi District, Uganda are as presented below. The phylotyping of *E. coli* based on the old Clermont method using *chuA, yjA* and Tspe4.C2 genes' analysis, revealed that these MDR strains of *E. coli* belongs to five phylo-groups namely: A, B1, B2, D and unknown (non-typeable) (Table 8 and Figures 1 to 3) while the new Clermont method showed that the MDR strains of *E. coli* belongs to seven phylo-groups namely: A, B1, B2, D, E, Clade1 and unknown (non-typeable) (Table 8 and Figures 1 to 4). The phylotyping of MDR *E. coli* strains based on the old Clermont method revealed that, most prevalent strains of MDR of *E. coli* belong to the phylo-group B2 with 12/31 (38.7%) followed by A 7/31 (22.6%), B1 5/31 (16.1%), D 5/31 (16.1%) and unknown 2/31 (6.5%) (Table 8 and Figures 1 to 4). The isolates assigned as group B2 9/12 (75.0%), B1 2/5 (40.0%) and A 2/7 (28.6%) by using the old Clermont method couldn't be phylotyped using the new Clermont method and were grouped as unknown or non-typeable strains of *E. coli* (Table 9). The contested results of phylogenetic groups; E or Clade 1, and D or E were screened with E specific primers (ArpAgpE) to obtain the exact *E. coli* phylogenetic group (Figure 5). All the isolates were subjected to the internal control specific primers (trpBA) for *E. coli* to ascertain species specificity of *E. coli* strains studied (Figure 6).

## DISCUSSION

This study determined the antimicrobial resistance profiles, multidrug resistance profiles, multiple antibiotic resistance indices (MARI), factors associated with MDR UTIs, and the phylogenetic groups of MDR *E. coli* strains isolated from the urinary tract among patients attending hospitals in Bushenyi District, Uganda. This study provides an essential baseline information, that calls for continues monitoring of antimicrobial resistance (AMR) profiles among bacterial uropathogens, to provide reference guidelines to clinicians on the selection of the most suitable drugs in the treatment of UTIs. The increase of MDR among the bacterial pathogens in the world today poses a public health challenge in the management of infectious diseases (D'Andrea et al., 2011; Anwar et al., 2018).

Despite the fact that, some of the antimicrobial agents in the study demonstrated substantial sensitivities, some uropathogens showed extremely high level of AMR, more especially to erythromycin 74/86 (86.0%). The Gram negative bacteria demonstrated 56/56 (100.0%) resistance to erythromycin, this finding is more similar to previous reports by Kyabaggu et al. (2007); Kibret and Abera, (2011) and Adwan et al. (2014). The Gram positive bacteria resistance to erythromycin of 18/30 (60.0%) reported in this study is more comparable to previous report by Kabugo et al. (2016). The resistance of 74/86 (86.0%) to co-trimoxazole in this study is in agreement with previous reports of Odoki et al. (2015), 79/103 (76.7%); Ampaire et al. (2015), 12/14 (85.7%); Mwaka et al. (2011), 44/55 (80.0%) and Odongo et al. (2013), 60/82 (73.2%). The AMR of co-trimoxazole in this study is lower as compared to the finding of Katongole et al. (2015) who reported 51/53 (96.2%) and higher than

**Table 4.** Multiple Antibiotic Resistance Indices (MARI) of the bacterial uropathogens.

| Uropathogens | MARI | Antibiotics to which the isolates are resistant |
|---|---|---|
| *E. coli* | 0.9 | AMP, NI, CX, SXT, GEN, IMI, E |
| *K. pneumoniae* | 0.9 | AMP, NI, CX, SXT, GEN, IMI, E |
| *K. oxytoca* | 0.6 | AMP, NI, CX, SXT, E |
| *P. mirabilis* | 0.6 | AMP, NI, CX, SXT, E |
| *P. vulgaris* | 0.4 | CX, SXT, E |
| *S. aureus* | 1.0 | AMP, NI, CX, SXT, AK, GEN, IMI, E |
| *E. faecalis* | 0.8 | AMP, NI, SXT, E |

AMP=ampicillin; NI=nitrofurantoin; CX=cefoxitin; SXT=co-trimoxazole; AK= amikacin; GEN=gentamicin; IMI=imipenem; E=erythromycin.

**Table 5.** Bivariate analysis between socio-demographic variables and MDR UTIs.

| Variable | Categories | Unadjusted OR | 95% CI | p-value |
|---|---|---|---|---|
| Department | In-patients | 3.616 | 1.017-12.860 | 0.047 |
| | Out-patients | 1 | | |
| Age | ≤19 years | 0.529 | 0.124-2.261 | 0.390 |
| | ≥20years | 1 | | |
| Gender | Female | 2.417 | 0.690-8.463 | 0.168 |
| | Male | 1 | | |
| Residence | Rural | 1.463 | 0.415-5.160 | 0.554 |
| | Sub-urban | 0.564 | 0.065-4.894 | 0.604 |
| | Urban | 1 | | |
| Marital status | Married | 1.964 | 0.516-7.471 | 0.322 |
| | Single | 1.226 | 0.124-12.120 | 0.861 |
| | Others | 1 | | |
| Level of education | No education | 0.793 | 0.205-3.075 | 0.737 |
| | Primary | 0.939 | 0.180-4.900 | 0.941 |
| | Secondary | 0.527 | 0.061-4.591 | 0.560 |
| | Tertiary | 1 | | |
| Sexual intercourse | Yes | 1.160 | 0.269-5.006 | 0.843 |
| | No | 1 | | |

CI=confidence interval, p=probability, OR=odds ratio, p≤0.05 value is statistically significant under logistic regression.

the finding of Ali et al. (2017) who reported 236/351 (67.2%). Furthermore the AMR of 69/86 (80.2%) to ampicillin observed in this study is inconformity with other previous studies of Odoki et al. (2015); Ampaire et al. (2015) and Katongole et al. (2015) who reported 90/103 (87.4%), 10/14 (71.4%) and 7/8 (87.5%) respectively. The resistance of ampicillin demonstrated in our study is lower than 318.7/351 (90.8%) reported by Ali et al. (2017) and higher than 35/58 (60.3%) reported by Mwaka et al. (2011). This could be due to the nature of the studied participants such as diabetes, elderly, pregnant women, HIV and infants used in this study which was probably prone to recurrent UTI and subsequent therapeutic usage of this drug previously which might have led to a higher resistance.

Some antimicrobial agents' demonstrated high

**Table 6.** Bivariate analysis between health condition and MDR UTIs.

| Variable | Categories | Unadjusted OR | 95% CI | p-value |
|---|---|---|---|---|
| Pregnancy | Yes | 0.459 | 0.103-2.060 | 0.310 |
| | No | 1 | | |
| Hypertension | Yes | 1.082 | 0.212-5.516 | 0.924 |
| | No | 1 | | |
| Genitourinary abnormalities | Yes | 0.747 | 0.229-2.440 | 0.629 |
| | No | 1 | | |
| Diabetes mellitus | Yes | 1.082 | 0.212-5.516 | 0.924 |
| | No | 1 | | |
| HIV | Yes | 1.905 | 0.223-16.292 | 0.556 |
| | No | 1 | | |
| Any antimicrobial agent use in the last 12 months | Yes | 5.175 | 1.007-26.605 | **0.049** |
| | No | 1 | | |
| Cephalosporins in the last 12 months | Yes | 2.619 | 0.778-8.813 | 0.120 |
| | No | 1 | | |
| Fluoroquinolones in the last 12 months | Yes | 2.727 | 0.770-9.661 | 0.120 |
| | No | 1 | | |
| Abortion | Yes | 1.043 | 0.111-9.800 | 0.971 |
| | No | 1 | | |
| Chronic respiratory disease | Yes | 2.865 | 0.589-13.939 | 0.192 |
| | No | 1 | | |
| Recurrent UTI | Yes | 0.747 | 0.229-2.440 | 0.629 |
| | No | 1 | | |
| Previous hospital admission | Yes | 1.069 | 0.262-4.353 | 0.926 |
| | No | 1 | | |
| Family history of UTI | Yes | 0.873 | 0.168-4.536 | 0.872 |
| | No | 1 | | |
| Previous UTI | Yes | 0.758 | 0.226-2.537 | 0.653 |
| | No | 1 | | |
| Wrong prescription | Yes | 2.800 | 0.552-14.191 | 0.214 |
| | No | 1 | | |

CI=confidence interval, p=probability, OR=odds ratio, p≤0.05 value is statistically significant under logistic regression.

sensitivity against the bacterial uropathogens tested. Imipenem demonstrated 77/86 (89.5%) sensitivity in our study. This finding is comparable with findings of Katongole et al. (2015); Prakash and Saxena, (2013) and Jehan et al. (2015) who reported 17/19 (89.5%), 131/155 (84.5%) and 388/421 (92.2%) sensitivities respectively.

**Table 7.** Factors associated with MDR UTIs using stepwise forward multiple logistic regression analysis.

| Variable | Categories | Adjusted OR | 95% CI | p-value |
|---|---|---|---|---|
| Department | In-patients | 3.947 | 1.050-14.831 | 0.042 |
| | Out-patients | 1 | | |
| Any antimicrobial agent use in the last 12 months | Yes | 6.004 | 1.046-34.454 | 0.044 |
| | No | 1 | | |

CI=confidence interval, p=probability, OR=odds ratio, p≤0.05 value is statistically significant under logistic regression.

**Table 8**. Phylo-groups in MDR strains of *E. coli* isolated from the urinary tract.

| Phylo-groups | Old Clermont method | New Clermont method |
|---|---|---|
| B2 | 12 (38.7) | 4 (12.9) |
| A | 7 (22.6) | 5 (16.1) |
| B1 | 5 (16.1) | 3 (9.7) |
| Unknown | 2 (6.5) | 15 (48.4) |
| D | 5 (16.1) | 2 (6.6) |
| E | - | 1 (3.2) |
| Clade I | - | 1 (3.2) |
| Total | 31 (100.0) | 31 (100.0) |

"-"=no phylo-group found.

The sensitivity shown by imipenem in our study is slightly lower than 319/351 (98.6%) reported by Ali et al. (2017). This study also found out a high sensitivity of bacterial uropathogens to amikacin 74/83(89.2%). The high sensitivity of amikacin in this study is similar to 324.7/351 (92.5%) sensitivity reported by Ali et al. (2017). The sensitivity shown by amikacin in this study is slightly higher than 273/421 (64.8%) and 106/155 (68.4%) previously reported by Jehan et al. (2015) and, Prakrash and Saxena, (2013) respectively. Furthermore, this study showed high sensitivity of bacterial uropathogens 69/83(83.1%) to gentamicin. The sensitivity of gentamicin demonstrated in this study is supported by previous study by Odongo et al. (2013). The sensitivity shown by gentamicin in this study is slightly lower than 242.5/351 (69.1%) and 11/18 (61.1%) previously reported by Ali et al. (2017) and Kabugo et al. (2016) respectively. Finally, this study demonstrated considerable sensitivity of 69/86 (80.2%) of nitrofurantoin to the bacterial uropathogens tested. This substantial sensitivity of nitrofurantoin shown in this study is in conformity with previous reports of Odoki et al. (2015) and Ekwealor et al. (2016) who reported 78/103 (75.7%) and 191/212 (90.1%) sensitivities respectively. The sensitivity shown by nitrofurantoin in our study is in agreement with study done by Kabugo et al. (2016) who reported 12/18 (66.7%) sensitivity of uropathogens to nitrofurantoin.

This study demonstrated that the prevalence of MDR strains observed in the *Klebsiella spp.* is higher than previous reports (Regmi et al., 2018; Mahato et al., 2018; Shabbir et al., 2018). This could be due to the high use of over the counter antibiotics, bought from unlicensed drug stores and in open markets and self-medication in Uganda (UNAS et al., 2015). The high prevalence of MDR strains in *Proteus spp.* is in agreement with previous study by Mahato et al. (2018). Lastly, the high prevalence of the MDR strains in *E. coli* and *S. aureus* in this study is in conformity with reports by Regmi et al. (2018) and Mahato et al. (2018) respectively. The high prevalence of MDR strains in *E. coli* in this study is of high concern, since *E. coli* is the most common cause of bacterial UTIs (Tibyangye et al., 2015 and Odoki et al., 2019).

When the bacterial uropathogens were subjected to eight different antimicrobial agents, they all showed a MARI of ≥0.2. Multiple antibiotic resistance index is a method used to evaluate the extent to which a particular bacterial population have attained AMR (Ugwu et al., 2009 and Chika et al., 2017). Multiple antibiotic resistance index value of ≥0.2 shows that this particular population of bacteria arose from an environment where various antimicrobial agents have been used and abused (Ehinmidu, 2003; Tambekar et al., 2006; Oli et al., 2013). This is an obvious indication that a large proportion of bacterial isolates from the urinary tract were exposed to several antimicrobial agents that have led to the significant increase in AMR. Related incidences of resistance have been reported elsewhere, though to

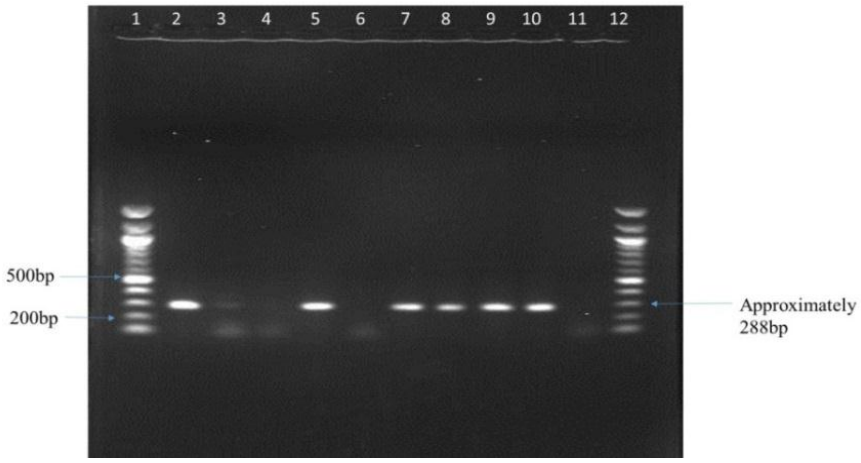


**Figure 1.** Gel images showing the *chuA* gene. Lanes 1 and 12 contain Markers of Molecular Weight (100 bp DNA ladder, New England Biolabs (NEB), USA). Lanes 5,7,8,9 and 10 are positive for the *chuA* gene. Lanes 3, 4 and 6 are negative for the *chuA* gene. Lanes 2 and 11 contain positive and negative controls respectively.

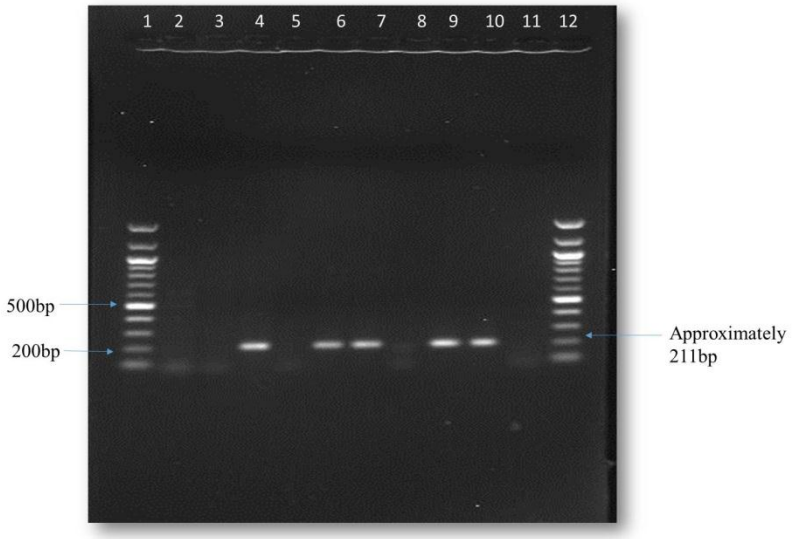


**Figure 2.** Gel images showing the *yjA* gene. Lanes 1 and 12 contain markers of molecular weight (100 bp DNA ladder, New England Biolabs (NEB), USA). Lanes 4, 6, 7 and 9 are positive for the *yjA* gene. Lanes 2, 3, 5 and 8 are negative for the *yjA* gene. Lanes 10 and 11 contain positive and negative controls respectively.

different sets of antimicrobial agents (Ehinmidu, 2003; Ekwealor et al., 2016). In addition, reports of bacterial uropathogens' resistance to frequently used antimicrobial agents have been documented (Prakash and Saxena, 2013).

This study demonstrated that hospitalization bears statistically significant association (OR=3.947; 95% CI: 1.050-14.831; p<0.05) with MDR UTIs. Our findings are in agreement with previous report by Tenney et al. (2017). Hospital environment is known to be a reservoir for MDR organisms and these antibiotic resistant organisms are found in high-touch surfaces in hospital wards or patients admission rooms, for example bedside tray tables, bed controls and call buttons (Dancer , 2014). These nosocomial MDR organisms includes: methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), and resistant Gram-negative bacteria. The resistant Gram-negative bacteria that cause UTIs

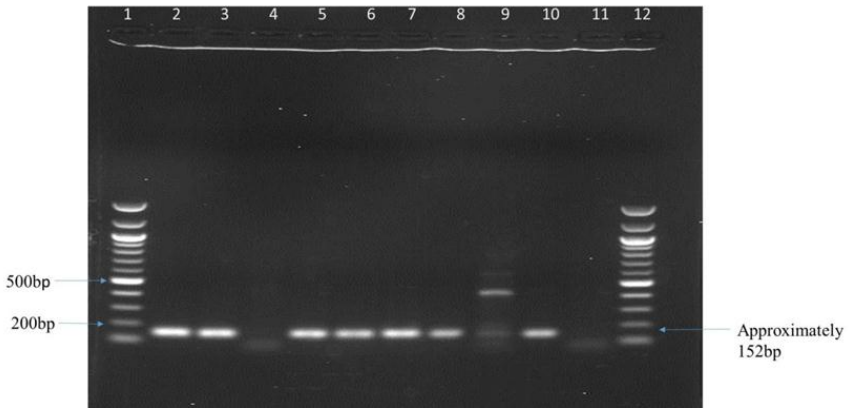


**Figure 3.** Gel images showing the Tspe4.C2. Lanes 1 and 12 contain markers of molecular weight (100bp DNA ladder, New England Biolabs (NEB), USA). Lanes 3,5,6,7,8 and 10 are positive for Tspe4.C2. Lanes 4 and 9 are negative for Tspe4.C2. Lanes 2 and 11 contain positive and negative controls respectively.

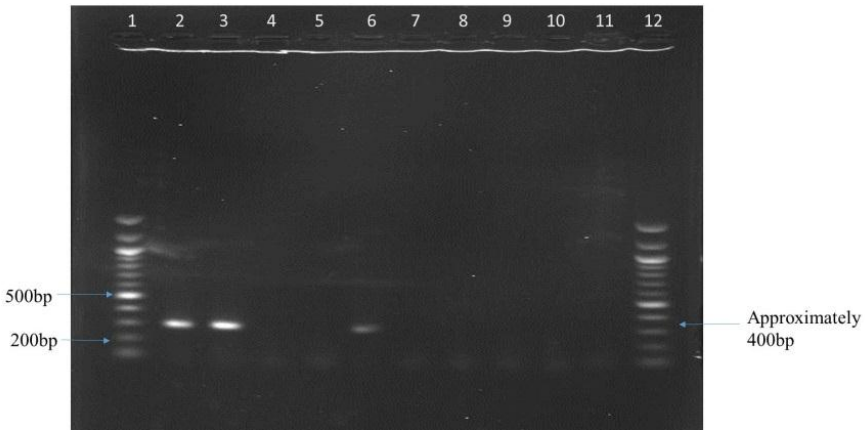


**Figure 4.** Gel images showing the *arpA* gene. Lanes 1 and 12 contain markers of molecular weight (100bp DNA ladder, New England Biolabs (NEB), USA). Lanes 3 and 6 are positive for *arpA* gene. Lanes 4,5,7,8,9 and 10 are negative for the *arpA* gene. Lanes 2 and 11 contain positive and negative controls respectively.

arise from the human gut flora (Sarowska et al., 2019; Kalluru et al., 2018). Patients and staffs harboring these MDR organisms potentially contaminates high-touch surfaces in hospital wards or patients' admission rooms, hence increasing the risk of infecting other patients, hospital staffs and visitors that may come into contact with these contaminated surfaces (Dancer , 2014; Nseir et al., 2011).

Furthermore, previous use of any antimicrobial agent in the last 12 months was found to have a statistical significant relationship (OR=6.004; 95% CI: 1.046-34.454; $p<0.05$) with MDR UTIs. This finding is supported by a previous study (Khawcharoenporn et al., 2017). The key factor involved in the development and spread of antimicrobial resistance is prior exposure to antimicrobial agents. There is a selection of a small fraction of cells from the wild type population that will be resistant to the antimicrobial agent being used during clinical treatment. This subpopulation of bacteria is selected to survive through spontaneous mutation that impedes antimicrobial action (Raymond, 2019; Cantón and Morosini, 2011).

The high prevalence of MDR strains in *E. coli* in this study is of high concern, therefore there is need to understand the genetic relatedness between the MDR strains in order to compare their genetic diversity with already documented strains. According to the old Clermont method, phylotyping of *E. coli* was based on the *chuA, yjA* and Tspe4.C2 genes' analysis and this revealed that, the high prevalence of the B2 phylo-group in UTIs is in agreement with previous report by Iranpour et al. (2015). Previous studies globally, have shown that pathogenic strains of invasive *E. coli* mainly are

**Table 9.** Number of *E. coli* isolates and the alterations/confirmations in phylo-groups designated by old and new Clermont methods.

| Old Clermont genotypes | | New Clermont genotypes | | | |
|---|---|---|---|---|---|
| **Clermont genotypes** | **Phylo-group** | **Clermont genotypes** | **Phylo-group** | **No. of isolates (%)** | **Alteration of old and new Clermont methods of genotypes** |
| - - - | A | + - - - | A | 5 | 0 |
| - - - | A | - - - - | Unknown | 2 | A to unknown |
| + - + | D | - + - + | B2 | 3 | D to B2 |
| + - + | D | + + - + | D | 2 | 0 |
| + + + | B2 | + + + + | Unknown | 9 | B2 to unknown |
| + + - | B2 | + + + - | Clade1 | 1 | B2 to Clade1 |
| + + - | B2 | + + + - | E | 1 | B2 to E |
| + + + | B2 | - + + + | B2 | 1 | 0 |
| - - + | B1 | + - - + | B1 | 3 | 0 |
| - - + | B1 | - - - + | Unknown | 2 | B1 to unknown |
| - + + | Unknown | + - + + | Unknown | 1 | Unknown to unknown |
| - + + | Unknown | - - + + | Unknown | 1 | Unknown to unknown |

"+"=positive for gene used in Clermont phylotyping; "-"=negative for gene used in Clermont phylotyping.

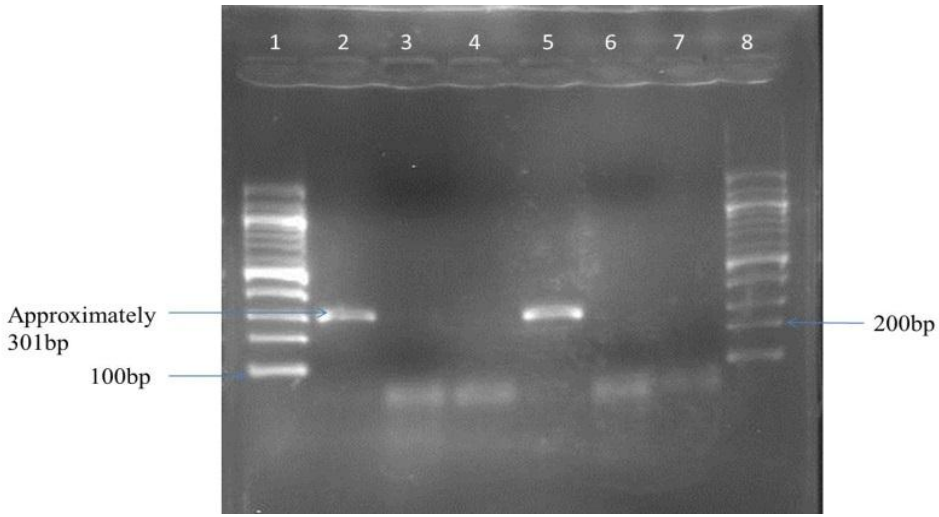


**Figure 5.** Gel images showing the *arpA* gene. Lanes 1 and 8 contain markers of molecular weight (100 bp DNA ladder, New England Biolabs (NEB), USA). Lane 5 is positive for the *arpA* gene. Lanes 3, 4 and 6 are negative for the *arpA* gene. Lanes 2 and 7 contain positive and negative controls respectively.

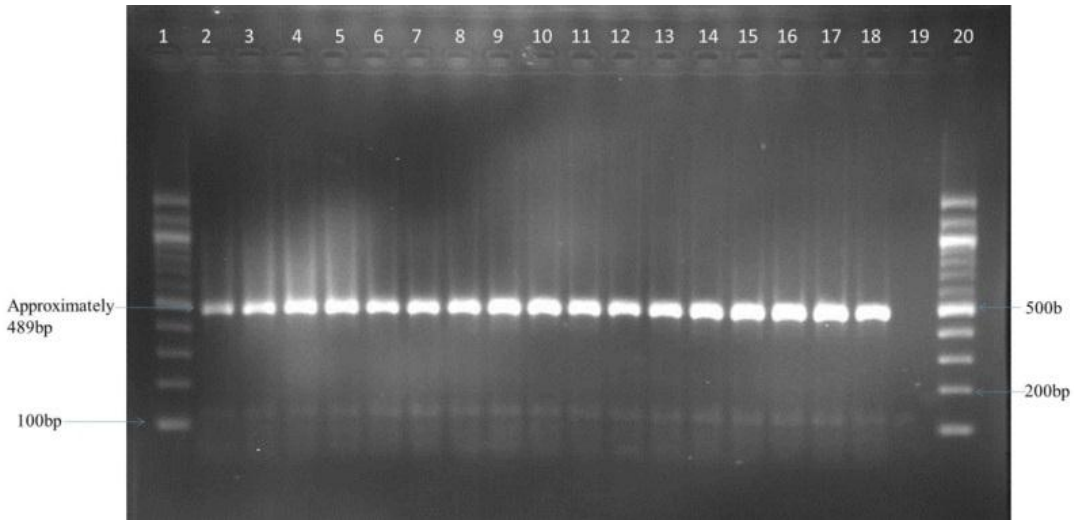


**Figure 6.** Gel images showing the *trpA* Internal control. Lanes 1 and 20 contain Markers of Molecular Weight (100bp DNA ladder, New England Biolabs (NEB), USA). Lanes 3-18 are positive for *trpA* gene. Lanes 2 and 19 contain positive and negative controls respectively.

composed of group B2 than group D. While group A or group B are mostly commensal strains (Basu et al., 2013; Ejrnaes, 2011). The new Clermont method revealed that the isolates assigned as phylo-group B2 9/12 (75.0%), B1 2/5 (40.0%) and A 2/7 (28.6%) by using the old Clermont method could not be phylotyped, consequently they were grouped as unknown or non- typeable strains of MDR *E. coli*. This kind of alteration of the phylogenetic groups of *E .coli* from old Clermont to the new Clermont method was also reported by Müştak et al. (2015), although to different sets of phylogenetic groups.

In 2013 Clermont and his colleagues were unable to detect these phylogenetic groups (- - - +, - - + +, + - + + and + + + +) using the new Clermont method, despite the enormous work they did to screen over 1000 *E. coli* strains (Clermont et al., 2013). In 2015 Iranpour and colleagues were able to detect two of these novel isolates belonging to phylogenetic groups (+ - + + and + + + +) in *E. coli* isolated from the urinary tract (Iranpour et al., 2015). Surprisingly, this study was able to find two more novel isolates belonging to the phylogenetic groups (- - - +, - - + +) which was not detected by Clermont and colleagues in 2013 using the new Clermont method (Clermont et al., 2013). The isolates designed as unknown or non-typeable strains of MDR *E. coli* are to be subjected to MLST as recommended by Clermont and colleagues in 2013 to delineate their phylogenetic group identities.

Our study had the following limitations: insufficient data on the previous patients' antimicrobial use as some of them did not have records of previous medications. Also, we did not distinguish, recurrent, uncomplicated and complicated UTIs. Therefore the resistance pattern in these subjects could not be attained. Finally, we were unable to afford a modern DNA sequencing-based approach which is the gold standard for genotyping bacteria.

## Conclusion

Erythromycin, co-trimoxazole and ampicillin were the most resisted antimicrobial agents studied. The significant increase in the values of MARI and the high prevalence of MDR strains in *E. coli*, which is the leading cause of UTIs is of utmost concern. Multiple logistic regression revealed that hospitalization and previous use of any antimicrobial agent in the last 12 months bear statistically significant associations with MDR UTIs. The new Clermont method revealed that the isolates assigned as phylo-group B2 9/12 (75.0%), B1 2/5 (40.0%) and A 2/7 (28.6%) by using the old Clermont method couldn't be phylotyped, consequently they were grouped as unknown or non-typeable strains of MDR *E. coli*. To prevent the development of MDR among the bacterial uropathogens, this study therefore recommend antimicrobial susceptibility testing of the common bacterial uropathogens implicated in UTIs before antimicrobial agents prescription by clinicians among patients of the following category: hospitalized and patients with history of previous use of any antimicrobial agent in the last 12 months. For empiric treatment of UTIs in Bushenyi District, nitrofurantoin still remains the first line of choice. Although the Old and the new Clermont method is accepted for phylotyping of *E. coli,* this study recommends the use of modern DNA sequencing-based approaches which is the gold standard for genotyping bacteria, that this current study couldn't afford.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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