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In-vitro Antimicrobial Susceptibility Pattern of Isolates from Urine in Butembo, Democratic Republic of the Congo

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Abstract

Objective: This study aimed to determine the isolates from urine cultures at patients suspected with urinary tract infection and their *in-vitro* susceptibility pattern to antimicrobials at the Central Laboratory of Research of the "Université Catholique du Graben" (UCG) in Butembo

Methodology: This was a cross-sectional study adopting a descriptive approach, conducted from January, 2015 to December, 2016. Six hundred and seventeen patients were screened. Freshly voided mid- stream urine sample were taken according to standard method. Isolation of the microbial agents of urinary tract infections was done on blood agar and MacConkey agar media. The culture was repeated in contrast with significant bacteria and urinalysis if the isolate at the first culture is known non-pathogenic. The antimicrobial susceptibility testing was done by using disk diffusion (Kirby Bauer's) technique.

Results: Out of the 617 patients screened, the culture was positive in 77.3% (477 cases). The most isolated bacteria from urine culture were *Staphylococcus aureus* (47%), *Streptococcus spp* (12%), *Escherischia coli* (10.9%), *Moraxella spp* (7.5%) and *Bacillus spp* (2.3%). Antibiotics which were sensible to three or more isolates were ciprofloxacin, cefuroxime, cefotaxime and vancomycin. It was also observed that all the bacterial species have a Multiple Antibiotic Resistance Index greater than 0.2.

Conclusion: Continuous monitoring of antimicrobial susceptibility pattern of bacterial isolates implicated in urinary tract infections is needed seeing the high Multiple Antibiotics Resistance Indices of all samples isolates. And this should be prior to antibiotic prescription in order to ensure an optimal, desired and cost-effectiveness treatment. For empiric treatment of urinary tract infections in Butembo, we suggested ciprofloxacin, cefuroxime and cefotaxime as the first line antibiotics of choice.

Keywords: Antibiotics; Democratic Republic of the Congo; Resistance; Urinary tract infections

Introduction

The growth of resistant bacteria to commonly used antimicrobials became a concern of the worldwide public health [1,2]. The expansion of antimicrobial resistance (AMR) does not only entail an increase of morbidity and mortality but constitutes also to a heavy economic burden. The health systems in countries with weak income and that fight against the lack of financing and the faltering institutions are particularly hit by AMR [3-5].

The rational use of antimicrobials has an incontestable advantage, but physicians and the public use them irrationally [6,7]. In developing countries, the development and propagation of AMR are increasing because of irrational use of antimicrobials, the maladjusted or non-existent programs fighting against infections, bad quality drugs, weakness of laboratory capacity and deficiencies of surveillance and insufficiencies of the regulation on the use of antimicrobials [3]. In the Democratic Republic of the Congo (DRC), the availability of antimicrobials in free-market that means even without medical prescription is one of the reasons aggravating the expansion of the AMR [4]. The uncontrolled and irrational use of folk medicine is also among the reasons [8].

Multidrug resistant (MDR) pathogens propagate not only locally, regionally but also globally, with new pathogens introduced recently that spread quickly at the susceptible hosts [9]. So, surveillance data needed to be gathered and of sentries centers selected. The local epidemiology of AMR should constantly be updated to detect some changes in the causal pathogens and their sensibility to different antimicrobials.

The urinary tract infections (UTI) constitute the second motive of consultation and prescription of antimicrobials in the physician's office and in emergencies services in France [10]. While the UTI are well documented worldwide [11-13], there are not any national reports currently on the prevalence of UTI in DRC. Nevertheless, there are some regional reports. Thus, Irenge et al. [14] found in the South-Kivu a rate of AMR of 21.1% among hospitalized patients and 8.4% among out-patients. The bacteria more met were *Escherichia coli*, *Klebsiella spp*. and *Enterobacter spp*.

In Butembo, the bacteria responsible for the UTI are mainly the *Staphylococcus aureus* (57.2%), *Escherischia coli* (4.71%), *Streptococcus* (4.13%) and *Moraxella* (2.73%) [15], but no survey has been done for determining their sensitivity to antimicrobials.

The knowledge of bacteria causing the UTI and their sensitivity to antimicrobials is necessary to assure an appropriate treatment. Therefore, this study aimed to determine isolates from urine of patients with UTI and their sensitivity to antimicrobials at patients attending the Central Laboratory of Research of the "Université Catholique du Graben" (UCG) in Butembo.

Material and Methods

Study design

This was a cross-sectional study adopting a descriptive approach, conducted from January 2015 to December 2016 in the department of microbiology at the Central Laboratory of Research of the "Université Catholique du Graben" (UCG) in Butembo.

Population and sample size

The population study was constituted by patients with suspicion of urinary tract infection (UTI) attending the Central Laboratory of Research of the UCG for urine culture. Patients living out of Butembo, those whose urine samples were sent to the laboratory by hospitals, those who were taking antimicrobials, and those who did not consent to the study were excluded from this study. Furthermore, cultures showing fungal growth, mixed growth and those not conclusive in the laboratory, irrespective of reason, were excluded.

The sample size was exhaustive including all patients who met the inclusion criteria. Convenience selection of urine culture reports was employed as a sampling method. Thus, 617 patients were retained for this study.

Specimen collection

Freshly clean-voided mid-stream urine sample were taken according to standard method [16]. It was collected in a sterile wide mouth container. All the urine samples were processed within one hour after the collection for aerobic bacterial culture. If delayed, samples were refrigerated and processed within 4-6 hours.

Specimen processing

Urine specimen was cultured for isolation of the microbial agents of UTI on blood agar and MacConkey agar media. A measured amount of urine, using calibrated loop method was inoculated to nutrient agar medium for colony count. Equal or more than 10^5 CFU/ml of a single potential pathogen or for each of two potential pathogens interpreted as positive UTI and a result of 10^2-10^4 CFU/ml was repeated. A less than 10^2 CFU/ml was interpreted as negative UTI. An organism known as normal flora were considered as pathogen if after the first culture, the repeated second culture, where sample were taken carefully, isolated the same organism, with a contrast with significant bacteriuria and in conjunction with the urinalysis results. All the bacteria isolated from urine in this study were identified using conventional biochemical tests [17].

Antimicrobial susceptibility study

The antimicrobial susceptibility testing was done on Mueller-Hinton Agar using disk diffusion (Kirby Bauer's) technique. This method was done according to Clinical Laboratory Standard Institute guidelines to determine susceptibility of UTI agents. The antibiotic disks comprised, oxacilline (1 µg), amoxicillin-clavulanic acid (augmentin) (20+10 µg), cefuroxime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), meropenem (10 µg), vancomycin (30 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (30 µg), spectinomycin (100 µg), chloramphenicol (30 µg), doxycycline (30 µg), erythromycin (15 µg), azithromycin (15 µg), clarithromycin (15 µg), clindamycin (2 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg) and levofloxacin (5 µg) [18]. Microbial with intermediate susceptibility was considered as resistant for data analysis.

The Multiple Antibiotic Resistance Indices (MARI)

The MARI calculation was done by dividing the number of antibiotics to which a microorganism is resistant by the total number of antibiotics to which the organism was subjected to. Bacteria with a MARI greater than 0.20 implies that the resistance strains of such bacteria are originated from an environment where several antibiotics are used or misused.

Data analysis

We used the software WHONET 5.6 for data analysis. Results were expressed as counts and percentages. Association of variables was analysed by using odds ratio (OR), with a confidence interval of 95% (95% CI). We considered associations as significant when OR were greater than 1 with a P-value of less than 0.05.

Ethical consideration

Ethical clearance (CE-UCG/FACMED/001-2015) was obtained from the local research ethics committee of the Faculty of Medicine at the "Université Catholique du Graben". Patients were explained and adequately informed on the purpose of the study and were assured of privacy and confidentiality. A consent form was signed by the patient who accepted participation to the study. For patients under 18 years, the consent was signed by their parents or guardians.

Results

Out of the 617 patients screened, the urine culture is positive in 77.3% (477 cases). The urine culture proved to be positive in 63.7% of females and the age group of 26-35 is the more touched (44.2%) but statistically the age group of 6-15 is more touched (OR=0.3, IC: 0.1-0.7 and P=0.003) (Table 1). The Figure 1 shows that Gram positive bacteria are isolated in 62.9% and Gram negative bacteria in 37.1% (Figure 1). Following their frequency, the most isolated bacteria from urine culture are *Staphylococcus aureus* (47%), *Streptococcus spp* (12%), *Escherischia coli* (10.9%), *Moraxella spp* (7.5%) and *Bacillus spp* (2.3%) (Table 2). The Table 3 shows that *Staphylococcus aureus* was resistant to all antibiotics tested except for cefuroxime, clindamycin, ciprofloxacin levofloxacin, doxycycline, neomycin and vancomycin to which it was sensible. *Streptococcus spp*. was resistant to all antibiotics tested except for ceftriaxone, cefotaxime, ciprofloxacin and vancomycin. *Escherischia coli* were only sensitive to ceftriaxone, kanamycin, spectinomycin, azythromycin and ciprofloxacin. The MARI of all bacterial isolates was greater than 0.20 (Table 4).

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Variables	**Results of urine culture**		**OR**	**95% IC**	**P-value**
	Positive, n (%)	**Negative, n (%)**			
Sex					
Female	304 (63.7)	83 (59.3)	1.2	0.8-1.8	0.339
Masculine	173 (36.3)	57 (40.7)	0.8	0.6-1.2	
Total	477 (100)	140 (100)	-	-	-
Age (years)					
≤ 5	13 (2.7)	3 (2.1)	1.3	0.3-5.74	0.703
6	8 (1.7)	9 (6.4)	0.3	0.1-0.7	0.003
16-25	67 (14.1)	19 (13.6)	1	0.6-1.9	0.887
26-35	211 (44.2)	65 (46.5)	0.9	0.6-1.4	0.646
36-45	113 (23.7)	28 (20.0)	1.2	0.8-2.0	0.361
≥ 46	65 (13.6)	16 (11.4)	1.2	0.7-2.3	0.498
Total	477 (100)	140 (100)	-	-	-

Table 1: Socio-demographic characteristics of patients.

Bacterial isolates	**n (%)**
Staphylococcus aureus	224 (47,0)
Streptococcus spp	57 (12,0)
Escherichia coli	52 (10,9)
Moraxella spp	36 (7,5)
Bacillus spp	11 (2,3)
Erwina spp	9 (1,9)
Listeria spp	8 (1,7)
Edwardsiella hoshinae	6 (1,3)
Yersinia frederiksenii	6 (1,3)
Pneuteura spp	6 (1,3)
Cedecea lopagei	5 (1,1)
Flavobacterium odoratum	5 (1,1)
Aeromonas hydrophila	4 (0,8)
Klebsiella pneumoniae	4 (0,8)
Ledercia adercaboxylata	3 (0,6)
Salmonella spp	3 (0,6)
Citrobacter freundii	3 (0,6)
Pseudomonas spp	2 (0,4)
Klebsiella rhinoscleromatis	2 (0,4)

Budvicia aquatica	2 (0,4)
Escherichia vulneris	2 (0,4)
Neisseria gonorrheae	2 (0,4)
Piesiomonas shigeloides	2 (0,4)
Cirobacter diviersys	2 (0,4)
Others*	21 (4,4)
Total, n (%)	477 (100)

*Others : *Enterobacter agglomeran, Enterobacter amorigenus, Enterobacter cloacea, Escherichia fergusonii, Ewingili americana, Pasteurella aerogenes, Proteus penneri, Pseudomonas aeruginosa, Rnanella adualis, Salmonella typhi, Serratia ficana, Serratia liquefacens, Serratia marcescens, Vibrio hollisae, Yersinia enterocolitica, Cedecea neteri, Klebsiella ozaenae, Moellerella*

Table 2: Incidence of bacterial isolates from urine samples of the patients.

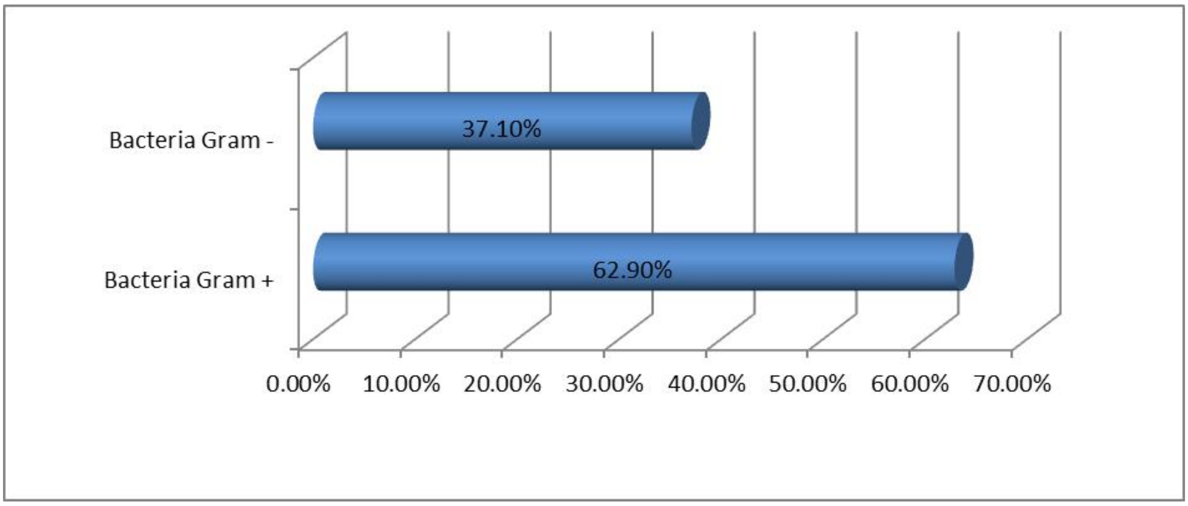

Figure 1: Repartition of bacteria according to their Gram Staining.

Antibiotics	*S. aureus*		*Streptococcus spp*		*E. coli*		*Moraxella spp*		*Bacillus spp*	
	S	R	S	R	S	R	S	R	S	R
Augmentin	31 (18.4)	137 (81.6)	17 (40.5)	25 (59.5)	2 (5.9)	32 (94.1)	5 (25.0)	15 (75.0)	1 (10.0)	9 (90.0)
Oxacilline	2 (3.0)	64 (97.0)	2 (14.3)	12 (85.7)	0 (0.0)	3 (100)	2 (50.0)	2 (50.0)	0 (0.0)	1 (100)
Meropeneme	14 (40.0)	21 (60.0)	2 (25.0)	6 (75.0)	8 (44.4)	10 (55.6)	3 (75.0)	1 (25.0)	0 (0.0)	1 (100)
Cefuroxime	28 (63.6)	16 (36.4)	6 (40.0)	9 (60.0)	4 (33.3)	8 (66.7)	3 (50.0)	3 (50.0)	2 (100)	0 (0.0)
Ceftriaxone	62 (37.6)	103 (62.4)	21 (56.8)	16 (43.2)	21 (52.5)	19 (47.5)	9 (42.9)	12 (57.1)	3 (33.3)	6 (66.7)
Cefotaxime	9 (29.0)	22 (71.0)	3 (60.0)	2 (40.0)	4 (44.4)	5 (55.6)	6 (60.0)	4 (40.0)	1 (100)	0 (0.0)
Vancomycin	36 (87.8)	5 (12.2)	8 (80.0)	2 (20.0)	0 (0.0)	1 (100)	1 (50.0)	1 (50.0)	1 (100)	0 (0.0)
Gentamycin	65 (49.2)	67 (50.8)	17 (37.8)	28 (62.2)	17 (44.7)	21 (55.3)	19 (61.3)	12 (38.7)	4 (100)	0 (0.0)
Spectinomycin	9 (20.9)	34 (79.1)	1 (8.3)	11 (91.7)	12 (80.0)	3 (20.0)	1 (33.3)	2 (66.7)	1 (100)	0 (0.0)

Neomycin	6 (50.0)	6 (50.0)	0 (0.0)	3 (100)	2 (40.0)	3 (60.0)	0 (0.0)	1 (100)	ND	ND
Kanamycin	0 (0.0)	5 (100)	0 (0.0)	1 (100)	1 (100)	0 (0.0)	ND	ND	ND	ND
Chloramphenicol	11 (43.8)	27 (56.2)	4 (33.3)	8 (66.7)	2 (33.3)	4 (66.7)	3 (50.0)	3 (50.0)	1 (33.3)	2 (66.7)
Doxycycline	109 (52.9)	97 (47.1)	24 (49.0)	25 (51.0)	10 (27.8)	26 (72.2)	26 (78.8)	7 (21.2)	3 (37.5)	5 (62.5)
Clindamycin	135 (65.2)	72 (34.8)	15 (27.3)	40 (72.7)	3 (6.4)	44 (93.6)	10 (30.3)	23 (69.7)	3 (30.0)	7 (70.0)
Erythromycin	50 (32.7)	103 (67.3)	10 (25.0)	30 (75.0)	2 (5.0)	38 (95.0)	5 (21.7)	23 (78.3)	1 (11.1)	8 (88.9)
Azithromycin	0 (0,0)	47 (100)	1 (7.1)	13 (92.9)	8 (50.0)	8 (50.0)	2 (50.0)	2 (50.0)	0 (0.0)	1 (100)
Clarithromycin	7 (15.2)	39 (84.8)	2 (14.3)	12 (85.7)	1 (6.7)	14 (93.3)	1 (50.0)	1 (50.0)	ND	ND
Ciprofloxacin	148 (64.3)	82 (35.7)	36 (63.2)	21 (36.8)	26 (52.0)	24 (48.0)	25 (67.6)	12 (32.4)	6 (54.5)	5 (45.5)
Levofloxacin	23 (53.5)	20 (46.5)	3 (23.1)	10 (76.9)	3 (20.0)	12 (80.0)	2 (40.0)	3 (60.0)	1 (100)	0 (0.0)
Nalidixic acid	4 (8.0)	46 (92.0)	0 (0.0)	7 (100)	8 (22.9)	27 (77.1)	22 (61.1)	14 (38.9)	0 (0.0)	2 (100)
S: n (%) Sensible, R: n (%) Resistant, ND: Not done										

Table 3: Antimicrobial susceptibility pattern of the bacterial isolates from urine specimens.

Isolates	MARI	Total number of antibiotic tested	Antibiotics to which the isolates are resistant
S. aureus	0.65	20	AMC, OXA, CRO, CTX, GEN, SPT, KAN, CHL, ERY, AZM, CLR, NAL and MEM
Streptococcus spp	0.8	20	AMC, OXA, CXM, GEN, SPT, NEO, KAN, CHL, DOX, CLI, ERY, AZM, CLR, LVX, NAL and MEM
E. coli	*0.75*	20	AMC, OXA, CTX, CXM, GEN, NEO, CHL, DOX, CLI, ERY, CLR, LVX, NAL, MEM and VAN
Moraxella spp	0.37	19	AMC, CRO, SPT, NEO, CLI, ERY and LVX,
Bacillus spp	0.59	17	AMC, OXA, CRO, CHL, DOX, CLI, ERY, AZM, NAL and MEM
AMC: Augmentin; OXA: Oxacilline; CRO: Ceftriaxone; CTX: Cefotaxime; CXM: Cefuroxime; GEN: Gentamycin; SPT: Spectinomycin; NEO: Neomycin; KAN : Kanamycin; CHL: Chloramphenicol; DOX: Doxycycline; CLI: Clindamycin; ERY: Erythromycin; AZM: Azithromycin;CLR: Clarithromycin; CIP: Ciprofloxacin; LVX: Levofloxacin; NAL: Nalidixic Acid (Negram); MEM: Meropeneme; VAN: Vancomycin			

Table 4: Multiple Antibiotic Resistance Indices (MARI) of the bacterial isolates.

Discussion

Urine cultures were positive in 63.7% of females and 36.3% of males as shown in Table 1. This result shows that, females are more vulnerable for the urinary tract infections (UTI) than males. These results are close to the ones of Bentroki et al. in their survey on the antibiotics resistance of isolated stumps of communal urinary infections between 2007 and 2011 in Guelma (Algeria). They had observed predominance of urinary infections in females in 85% [19].

In Bukavu (Democratic Republic of the Congo), Irenge et al. had also found a predominance of urinary infections in females with a sex ratio of 1.73 in favour of female [14]. Several authors [6,7] had found a predominance of UTI in women. It is explained by the passage of organisms from digestive tract to the genital apparatus toward the woman's urinary ways. It is facilitated by the short and large urethra in women. Men are protected from the urinary infections by the distance that separates the anus to the urinary meatus (the masculine urethra is long relatively about 16 centimetres) [6,7,15].

We observed that all age groups were touched by UTI but the age groups which were touched more were those between 16-25 years, 26-35 and 36-45 years respectively with 14.1%, 44.2% and 23.7%. These results are similar to those found by Kamwira in Butembo [15] and Irenge in Bukavu [14]. Akram, in a survey on etiologies and antibiotics resistance of communal UTI at Aligarh Hospital in India, found that the predominant age group was the one between 20-49 years with a prevalence of 51.04% [20]. The age interval of 16-25 years is the one during which there is adolescence crisis and the mindless sexuality seems to be high during this period. On the other hand, for ages lower than 15 years, the urinary infections would be due to the precariousness of hygiene.

The Gram positive bacteria were more isolated (62.9%) than the Gram negative bacteria (37.1%) (Figure 1). These results are different from those of other authors [6,7] who found a predominance of Gram negative bacteria. This would be explained by the fact that bacteria responsible of UTI are variable according to environment [15].

The isolates from urine culture were *Staphylococcus aureus* (47.0%), *Streptococcus spp* (12.0%), *Escherichia coli* (10.9%), *Moraxella spp* (7.5%), *Bacillus spp* (2.3%), *Erwina spp* (1.9%) and *Listeria spp* (1.7%) (Table 3). This distribution of bacterial isolates is not the same as the one reported in literature by other authors [14,15].

Irenge et al. found in their study on the antimicrobial resistance of isolates from urine cultures in South-Kivu, that the most frequent bacteria were Escherichia coli, *Klebsiella spp*. and *Enterobacter spp*. [14]. In Butembo, a survey conducted in 2014 on the pathogens responsible of UTI had shown that, the main bacteria were *Staphylococcus aureus* (57.2%), Escherischia coli (4.71%), Streptococcus (4.13%) and Moraxella (2.73%) [15]. In Europe, it is reported that, according to their frequency, the following pathogens are responsible for UTI: *Escherichia coli*, *Enterococcus*, *Pseudomonas aeruginosa*, *Klebsiella*, *Proteus mirabilis* and the *Enterobacter* [21]. This difference is due to the variation in the geographical distribution of the germs. This one can even be observed in the same hospital.

Staphylococcus aureus is the most frequently isolates from urine cultures in this study. It would be explained by the fact that the *Staphylococcus aureus* is ubiquitous germ finding in soil, air and water. It is also commensal germ of skin and the mucous membranes. It can be also found as normal flora in oropharynx and in stools. A third of individuals are carrier of *Staphylococcus aureus* in their nasal pits. The carelessness of hygiene is a factor that favors the infections bound to this species [22].

All isolated germs and tested for sensitivity showed that they are multi drug resistant (MDR). This study shows that *Staphylococcus aureus* and *Streptococcus spp* are resistant to penicillins, carbapenems, aminoglycosides, macrolides and cyclines. E. coli shows resistance to penicillins, carbapenems, cyclines and phenicols. Overall, the most concerning trends in Europe in 2013 were related to the occurrence of resistance in gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter* species) [22]. For *Escherichia coli* and *Klebsiella pneumoniae*, a continuous increase in resistance to key antimicrobial groups was noted. A majority of the isolates reported to European Antimicrobial Resistance Surveillance Network (EARS-Net) in 2013 was resistant to at least one of the antimicrobial groups under surveillance, and many of these showed combined resistance to third-generation cephalosporins, fluoroquinolones and aminoglycosides. Over the last four years (2010 to 2013), resistance to third-generation cephalosporins in *Klebsiella pneumoniae* and *Escherichia coli* increased significantly at European Union/European Economic Area (EU/EEA) level, as well as in many of the individual Members States. Many of the isolates resistant to third-generation cephalosporins were extended spectrum beta-lactamase (ESBL)-positive and showed resistance to additional antimicrobial groups. In addition, resistance to fluoroquinolones, aminoglycosides and carbapenems, as well as combined resistance to fluoroquinolones, third-generation cephalosporins and aminoglycosides increased significantly at EU/EEA level for *Klebsiella pneumoniae*, but not for *Escherichia coli* [22].

In Central Africa, the alarming rate of resistance has been noted for nearly all pathogens. Of special concern were MDR in *Shigella* and *Salmonella spp.* as well as the emergence of meticillin-resistant *Staphylococcus aureus* (MRSA), high-level penicillin-resistant *Streptococcus pneumoniae* and extended-spectrum ß-lactamases (ESBL) among Gram-negative pathogens [23].

According to the Multiple Antibiotics Resistance Indices, all bacterial isolates show a MARI greater than 0.20 (Table 4). This implies that a very large proportion of the bacterial isolates have been exposed to several antibiotics and thus have developed resistance to these antibiotics. Bunduki et al. [24] had already shown the irrational use of antibiotics among student in this area. In that study, antibiotics were used irrationally in 76.1% for treating UTI and the antibiotics most used were amoxicillin-clavulanic acid (Augmentin), penicillin, amoxicillin, ciprofloxacin, erythromycin, and doxycycline. This may expose bacteria to several antibiotics.

Conclusion

S. aureus, *Streptococcus pneumoniae* spp, *Escherichia coli*, *Moraxella spp* and *Bacillus spp* were the most prevalent among the uropathogens investigated. Continuous monitoring of antimicrobial susceptibility pattern of bacterial isolates implicated in UTI is needed seeing the high MARI of all samples isolates. And this should be prior to antibiotic prescription in order to ensure an optimal, desired and cost-effectiveness treatment. However, for empiric treatment of UTIs in Butembo, we suggested ciprofloxacin, cefuroxime and cefotaxime as the first line antibiotics of choice. Further studies have to be done for determining the phenotypic and genotypic resistance characterization of the multidrug resistant pathogens isolated in this study.

Conflicts of Interest Statement

All authors declare no competing interests regarding the publication of this paper.

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