Prevalence of Extended Spectrum Beta-Lactamase Producing Bacteria (ESBL) in Patients presenting with Urinary Tract Infections (UTIs) at a peri-Urban hospital, Uganda

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Research

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Abstract

Introduction Urinary tract infections remain one of the infections that are commonly diagnosed in Uganda with Enterobacteriaceae reported as the commonest uropathogens. Extended Spectrum Beta Lactamase producing Enterobacteriaceae have emerged as significant pathogens responsible for most of these urinary tract infections throughout the world. This resistance often leads to therapeutic failures from multidrug resistant bacteria. This study was carried out to determine the prevalence of Extended Spectrum Beta-Lactamase producing bacteria (ESBLs) in patients presenting with Urinary Tract Infections (UTIs) at Mbarara Municipal Council Health Center IV, an urban hospital in Mbarara district, south western Uganda.

Methods This was a Cross sectional study in which a total of 158 non repetitive midstream urine samples were obtained from 158 participants during months of June to August, 2018. Samples were cultured on conventional media (MacConkey agar and Cysteine Lysine Electrolyte Deficient agar) using a standard wire loop of capacity 0.05ml, incubated at 37 °C for 24hrs. Only samples that showed significant growth (growth of pure colonies $\geq 10^5$ cfu/ml) on primary culture were considered for further testing. The isolates were identified basing on the colony appearance, Gram stain and different conventional biochemical tests. Phenotypic expression of ESBLs production was detected using the double disc synergy test using Amoxicillin, Ceftazidime, Amoxicillin-Clavulanic acid and Ceftazidime-Clavulanic acid.

Results The results show that 23% of the 158 participants had Urinary Tract infection. The most common uropathogens isolated were Staphylococcus aureus (64%) and Escherichia coli (17%). Phenotypic ESBLs was seen in 27.8% (10/36) of the uropathogens and was commonly seen among Escherichia coli, 60% (6/10) isolates. Others included; Pseudomonas species (8%), Enterobacter aerogenes (1.3%), Citrobacter freundii (0.6%) and coagulase negative staphylococcus (0.6%).

Conclusion This study revealed a fairly high prevalence of ESBL producing organisms in Mbarara Municipal Council Health Center IV and high levels of resistance to third generation cephalosporins.

Introduction

Bacterial Urinary tract infections are commonly treated and managed with beta lactam antibiotics. Beta lactamases remain the most contributing factor to beta lactam resistance, and their increasing prevalence. Infections with ESBL producing organisms have been associated with poor outcomes, delayed initiation of appropriate treatment and increased hospital stay and expense (1, 2).

Extended-spectrum β -lactamases (ESBLs) are the enzymes mainly produced by Gram negative bacilli that have the ability to destroy or break down penicillins containing an oxyimino group, one or more extended spectrum cephalosporins antibiotics (first, second and third generation) and aztreonam and render them ineffective (2, 3). ESBLs are often encoded by genes located on large plasmids and these also carry genes for resistance to other antimicrobial agents such as aminoglycosides, trimethoprim, sulphonamides, tetracyclines and chloramphenicol. In addition recent studies have demonstrated co-

transfer of Qnr determinant on ESBL producing plasmids conferring resistance to nalidixic acid with reduced susceptibility to fluoroquinolones, thus a very broad antibiotic resistance extending to multiple antibiotic classes is now a frequent characteristic of ESBL producing enterobacteria isolates(4)

ESBL-producing bacteria have been isolated in many parts of the world (5) and the prevalence of antimicrobial resistance among uropathogens has been increasing worldwide due to irrational use of antibiotics in practice (5, 6). A recent study in Uganda at Kabale Regional Referral Hospital (KRRH) found 89% of the uropathogens were reported as phenotypically ESBL-producers (7). Another study in a South African hospital, established 36% of K. Pneumoniae isolates were ESBL-producers. Outbreaks of infections due to Klebsiella strains resistant to third-generation cephalosporins have also been reported in Uganda, Nigeria and Kenya (8, 9). At Mbarara municipal hospital, antibiotic susceptibility testing is not done while urinary tract infection patients are diagnosed and treated clinically.

Thus there is limited data about the magnitude of urinary tract infections that are caused by ESBL producing bacteria at this facility. Therefore, understanding the prevalence of ESBL producing bacteria causing UTIs will help improve on management of UTIs in this facility.

2. Materials And Methods

2.1. Study site and settings

This was a Laboratory based descriptive-cross-sectional study on UTI symptomatic patients receiving services at the Mbarara Municipal Council Health center IV, a peri-urban hospital. The hospital is a local government based health center and receives over 100 pregnant women a day majority of whom are of low financial status. Microbiological analysis of the samples was done at the Microbiology Laboratory of the Mbarara University of Science and Technology.

2.2. Data collection methods

Socio demographic data and laboratory data was collected. Laboratory data was collected during laboratory test procedures and included presence of Urinary Tract Infection and presence of ESBL producing uropathogens while socio demographic data was collected using a questionnaire which included variables such as age, gender, occupation, education levels and the history of antibiotic usage. Patients were instructed to collect a clean catch midstream urine sample in a well pre-labeled clean urine collection container as per the instructions from the SOPs of urine sample collection(10).

2.3 Culture and identification of isolates

Fifty microliters of urine were evenly spread over the surface of sterile Cystine Lactose Electrolyte Deficient (CLED) agar (source:HiMedia Laboratories Pvt.Ltd) plate using a sterile glass rod, incubated for 18-24 hours at 37°C. Plates with significant number of colonies ($>10^5$ CFU/ ml) were further subjected to

identification. Identification of isolates was carried using conventional biochemical tests for uropathogens (11).

2.4 Phenotypic detection of ESBLs

Detection of ESBL production was achieved using amoxicillin (liofilchems.r.l Italy) and ceftazidime discs (oxoid, USA) and double-disk synergy (DDS) method. The antibiotics used were; *ceftazidime* (30µg), ceftazidime-clavulanic acid (30+10µg), amoxicillin-clavulanic acid (30+10µg) and amoxicillin (30 µg). These antibiotics were placed at a distance of 20mm from each other. The plates were incubated overnight at 37°C. An increase of at least 5mm in the zone diameter for ceftazidime-clavulanic acid versus the zone diameter with ceftazidime tested alone was used to confirm the presence of ESBLs. A similar interpretation criterion was used when amoxicillin-clavulanic acid versus amoxicillin alone was used, as recommended by the Clinical and Laboratory Standards Institute

2.5. Quality control

Control organisms (*Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922) were used for the quality control of testing methods. All data was double entered to check its correctness and approved standard operating procedures were followed throughout the testing process.

2.6. Data analysis

Data was entered into Excel software, cleaned and then exported to SPSS version 23 for analysis. All categorical data was summarized in proportions/frequencies and presented in form of tables and graphs.

3. Results And Discussion

3.1. Participant characteristics

One hundred fifty (158) patients were recruited in this study. Majority of the participants; were females, lived in urban areas, were educated, had a sexual partner and were in the age bracket of 13 to 33 years (Table 1).

3.2. Prevalence of UTI

Of the 158 participants, 36 (23%) were confirmed as having UTI (Fig 1). The most common isolates were *Escherichia coli* and *Staphylococcus aureus* (Table 2).

3.3. Prevalence of ESBLs

In this study 10 out of the 36 isolates (28%) were ESBL producing (Fig 2). ESBL production was most expressed by *Escherichia coli* (6/10) (Table 3).

Discussion

In this study, the proportion of participants who were confirmed to have urinary tract infection were 36 out of the 158(24%). This relatively low proportion of participants with UTI was probably due to the fact that there are different nonbacterial conditions that can manifest with UTI like symptoms. Our study prevalence correlates with the findings from different studies which include; in Uganda by (14) with a prevalence of (27%), (A Agrawal et al. 2017) in India with a prevalence of (29.4%), in Senegal by (15) with a prevalence of (26.7%), in India by (16) with a prevalence of (29.44%) and in West Bengal, India by (17) with a prevalence of (27.75%).

The proportion in this study was higher compared to those from different studies which include; in Uganda by (12) which was (10%) and in Bangui, Central African Republic by(18) which was (10.9%).

The proportion in this study was lower in comparison with the prevalence got from different studies which include; in West Africa where a study by (kolawole et al. 2010) showed (60%) and in Tertiary Care Hospital in Bangladesh by (L Sharmin et al. 2017) with a prevalence of (46.7%). This indicates there is a varied prevalence of urinary tract infection in different areas due to socio-economical differences but UTI is still a challenge to the current health care system.

The most common uropathogens that were isolated were *Staphylococcus aureus* and *Escherichia coli*. This was probably due to the fact that they are both normal flora and more pathogenic in humans as compared to other isolates and if introduced in the urinary tract they can cause UTI depending on one's immune status. Since most of the participants were female, the anatomical closeness of the anal opening and urethral opening together with poor hygienic practices may facilitate the introduction of the pathogens in the urinary tract. Other common uropathogens isolated in this study included; *Pseudomonas* species, *Enterobacter aerogenes*, *Citrobacter freundii* and coagulase negative *Staphylococcus*. Our findings are similar to those that were showed by a study in Gulu, northern Uganda by (19) and a study in Ethiopia by (20) which indicated that both *Staphylococcus aureus* and *Escherichia coli* were the most common uropathogens that caused UTI.

In this study, uropathogens that were Extended Beta Lactam Producers had a frequency of 10 isolates out of the 36 isolates (27.8%). ESBL production is increasingly becoming a problem especially due to the fact that gram negative bacteria have many resistance mechanisms, due to erroneous use of drugs in empirical treatment and lack of appropriate infection control strategies, which may result in increased prevalence of resistant organism in the community.

This proportion was lower than the 62% in the study done in Mulago by(9) and the 31.1% in Nepal by (A Shrestha et al. 2016). However, it was higher than the 13.5% in the study done in Taiwan by(21). This variation was probably due to the difference in the sample size and the nature of participants recruited.

In this study, all the ESBL producing bacteria were Gram negative bacteria with *Escherichia coli* having the highest proportion of 6 out of 10 isolates (60%), other ESBL producing uropathogens included;

Enterobacteraerogenes, Citrobacterfluendii and Pseudomonas species. The results of this study were different from the study done at Kabale Regional Referral Hospital by (7) where Klebsiella species predominated with 52%, followed by Escherichia coli (44%) but similar to those in the study done in Cameroon by (22) where Escherichia coli (50.9%) was the most common pathogen.

Conclusion

This study showed that indeed not all patients presenting with symptoms of urinary tract infection had a urinary tract infection. Most common uropathogens that were responsible for urinary tract infections at MMC H/C IV were *Staphylococcus aureus* and *Escherichia coli*.

A number of uropathogens isolated were ESBL producing, and all ESBL producing uropathogens were Gram negatives with *Escherichia coli* showing the highest proportion.

There is need for sensitization of the community and the health workers about urinary tract infections and the existence of drug resistance among uropathogens.

The laboratories need to be well equipped with necessary materials for proper diagnosis of urinary tract infection and detection of ESBL production. This will help in the management of ESBL producing uropathogens and UTI at large.

There is need for improving on laboratory surveillance systems to help in the monitoring of the emergence of Extended Spectrum Beta Lactam producing pathogens routinely.

Declarations

Ethics approval and consent

Ethical clearance was sought from Faculty of Medicine Research Committee of the Mbarara University of Science and Technology. Study also obtained permission from the Director Mbarara District Health Services (DHS) and the Executive Director of Mbarara Regional Referral Hospital (MRRH). All participants were consented before recruitment into the study. The information obtained from patients was treated with confidentiality. Patients who were confirmed for UTIs were channeled in the health care system for proper management

Competing Interests

The authors declare that they have no competing interests

Consent of Publication

Not applicable

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Availability of data and material

The datasets during and/or analysed during the current study available from the corresponding author on reasonable request

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References

1. Fennell J, Vellinga A, Hanahoe B, Morris D, Boyle F, Higgins F, et al. Increasing prevalence of ESBL production among Irish clinical Enterobacteriaceae from 2004 to 2008: an observational study. *BMC infectious diseases*. 2012;12(1):116.
2. Bradford PA. Extended-spectrum β -lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clinical microbiology reviews*. 2001;14(4):933-51.
3. Munita JM, Arias CA. Mechanisms of antibiotic resistance. *Microbiology spectrum*. 2016;4(2).
4. Pitout JD, Nordmann P, Laupland KB, Poirel L. Emergence of Enterobacteriaceae producing extended-spectrum β -lactamases (ESBLs) in the community. *Journal of Antimicrobial Chemotherapy*. 2005;56(1):52-9.
5. Hoban DJ, Lascols C, Nicolle LE, Badal R, Bouchillon S, Hackel M, et al. Antimicrobial susceptibility of Enterobacteriaceae, including molecular characterization of extended-spectrum beta-lactamase-producing species, in urinary tract isolates from hospitalized patients in North America and Europe: results from the SMART study 2009–2010. *Diagnostic microbiology and infectious disease*. 2012;74(1):62-7.
6. Grude N, Tveten Y, Kristiansen BE. Urinary tract infections in Norway: bacterial etiology and susceptibility. A retrospective study of clinical isolates. *Clinical microbiology and infection*. 2001;7(10):543-7.
7. Andrew B, Kagirita A, Bazira J. Prevalence of Extended-Spectrum Beta-Lactamases-Producing Microorganisms in Patients Admitted at KRRH, Southwestern Uganda. *International Journal of Microbiology*. 2017;2017.
8. Musoke RN, Revathi G. Emergence of multidrug-resistant gram-negative organisms in a neonatal unit and the therapeutic implications. *Journal of tropical pediatrics*. 2000;46(2):86-91.
9. Kateregga JN, Kantume R, Atuhaire C, Lubowa MN, Ndukui JG. Phenotypic expression and prevalence of ESBL-producing Enterobacteriaceae in samples collected from patients in various wards of Mulago Hospital, Uganda. *BMC Pharmacology and Toxicology*. 2015;16(1):14.
10. Kish L. Survey sampling. 1965.

11. Ampaire L, Butoto A, Orikiriza P, Muhwezi O. Bacterial and Drug Susceptibility Profiles of Urinary Tract Infection in Diabetes Mellitus Patients at Mbarara Regional Referral Hospital, Uganda. *British Microbiology Research Journal*. 2015;9(4):1-5.
12. Mwaka A, Mayanja-Kizza H, Kigonya E, Kaddu-Mulindwa D. Bacteriuria among adult non-pregnant women attending Mulago hospital assessment centre in Uganda. *African health sciences*. 2011;11(2).
13. Organization WH. Guidelines on standard operating procedures for microbiology. 2000.
14. Odoki M, Bazira J, Agwu E. Health-point survey of bacteria urinary tract infections among suspected diabetic patients attending clinics in Bushenyi district of Uganda. *Special Bacterial Pathogens Journal*. 2015;1(1):1-5.
15. Barry M, Diallo B, Kanté D, Diallo I. Antimicrobial susceptibility profile of community-acquired urinary tract infection in adults: A seven months prospective cross-sectional study in Dakar Town, Senegal. *African Journal of Urology*. 2017;23(2):166-71.
16. Gajamer VR, Singh AK, Pradhan N, Kapil J, Sarkar A, Tiwari HK. Prevalence and Antibigram Profile of Uropathogens Isolated from Symptomatic and Asymptomatic Female Patients with Urinary Tract Infections and its Associated Risk Factors: Focus on Cephalosporin. *Research & Reviews: Journal of Medical Science and Technology*. 2018;7(1):32-41.
17. Maji SK, Mandal PK, Panja C, Dolai TK, Samanta A, Kundu PK, et al. Prevalence and Antibacterial Susceptibility Pattern of Aerobic Bacteria Causing Urinary Tract Infection in Tribal Population in Rural Part of West Bengal, India. *Int J Curr Microbiol App Sci*. 2016;5(6):406-12.
18. Bercion R, Mossoro-Kpinde D, Manirakiza A, Le Faou A. Increasing prevalence of antimicrobial resistance among Enterobacteriaceae uropathogens in Bangui, Central African Republic. *The Journal of Infection in Developing Countries*. 2009;3(03):187-90.
19. Odongo CO, Anywar DA, Luryamamoi K, Odongo P. Antibigrams from community-acquired uropathogens in Gulu, northern Uganda-a cross-sectional study. *BMC infectious diseases*. 2013;13(1):193.
20. Seifu WD, Gebissa AD. Prevalence and antibiotic susceptibility of Uropathogens from cases of urinary tract infections (UTI) in Shashemene referral hospital, Ethiopia. *BMC infectious diseases*. 2018;18(1):30.
21. Kung C-H, Ku W-W, Lee C-H, Fung C-P, Kuo S-C, Chen T-L, et al. Epidemiology and risk factors of community-onset urinary tract infection caused by extended-spectrum β -lactamase-producing Enterobacteriaceae in a medical center in Taiwan: A prospective cohort study. *Journal of Microbiology, Immunology and Infection*. 2015;48(2):168-74.
22. Nzalie RN-t, Gonsu HK, Koulla-Shiro S. Bacterial etiology and antibiotic resistance profile of community-acquired urinary tract infections in a Cameroonian city. *International journal of microbiology*. 2016;2016.

Tables

Table 1: A table showing socio-demographic characteristics of the study participants

Variables, n=158	Frequency	Percentage
Sex		
Female	128	81
Male	30	19
Age in completed years		
13-33	116	73.4
34-53	32	20.3
54-73	10	6.3
Education status		
Formal education	97	61.4
Informal education	61	38.6
HIV status		
Negative	121	76.6
Positive	37	23.4
Area of residence		
Town	89	56.3
Rural	69	43.7
Number of sexual partners		
None	12	7.6
One	136	86.1
More than one	10	6.3
Marital status		
Single	28	17.7
Married	130	82.3

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Table 2: Common Uropathogen isolated

Isolates	Frequency	Percentages (%)
<i>Staphylococcus aureus</i>	101	64
<i>Escherichia Coli</i>	28	17
<i>Pseudomonas species</i>	12	08
Others	17	11

Key: Others: *Enterobacter aerogenes*, *Citrobacter fruendii* and *coagulase negative Staphylococcus*

Table 3: Distribution of ESBL producing organisms.

Isolate	Frequency	Percentage (%)
<i>Escherichia coli</i>	6	60.0
<i>Enterobacteraerogenes</i>	2	20.0
<i>Citrobacterfluendii</i>	1	10.0
<i>Pseudomonas species</i>	1	10.0
Total	10	100.0

Figures

Figure 1

Pie chart showing UTI prevalence among the study participants

ESBL PRODUCTION

Figure 2

A pie chart showing the percentage of ESBL production