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Prevalence and Antimicrobial Susceptibilities of Bacteria Isolated from Circumcised and Non-Circumcised Women with Urinary Tract Infections in Different Gynecological Clinics in Khartoum Locality, Sudan

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Urinary tract infections (UTIs) are one of the diseases that are widely spreading among women. A number of factors contribute to UTIs, including circumcision, which narrows the opening of the urinary system. This cross-sectional study was conducted from April 2019 to February 2021 to detect the frequency of antimicrobial-resistant bacteria isolated from circumcised women attending two Clinics, for Gyncology in Khartoum locality. Conventional methods were used for isolation, identification, and antimicrobial susceptibility testing. A total of 80 midstream urine samples (n = 80) were collected from all female eligible volunteers, of which 40 had been circumcised and 40 had not. The study investigated 80 females aged 7-70 years, with a mean of 29.3 + 13.1 SD. There were 16/40 (40%) circumcised women who were married and 23/40 (60%) single, whereas for non-circumcised women there were 7/40 (17.5%) married and 33/40 (82.5%) single. Among the circumcised patients, 34/40 (85%) had growths compared to 6/40 (15%) of the non-circumcised participants, and UTIs were significantly associated with circumcision (P=0.001). Circumcised females had a 32 times higher odd ratio (O.R) of UTIs than non-circumcised females. Escherichia coli was the most predominant isolate among circumcised and non-circumcised women (15(37.5%)). The isolated bacteria in circumcised women were moderately sensitive to Augmentin 22/34 (67.7%) and Gentamycin 20/34 (58.8%) compared to other antimicrobial agents; Ciprofloxacin 16/34(47.1%), Cefuroxime 12/34(35.3) and Amoxycillin 10/34 (29.4%) while all Gram negative rods were highly resistant to Nalidixic acid (100.0%). In contrast to non-circumised women; all isolated bacteria were highly sensitive to Gentamicin 6/6 (100.0%) and Cefuroxime 5/6(83.3%), and moderate sensitive to Augmentin 4/6 (66.7%) and Ciprofloxacin 4/6(66.7%). Also all isolated were highly resistant to Nalidixic acid (100.0%) and Amoxycillin 1/6 (16.7%). UTIs and antimicrobial-resistant bacteria were more prevalent among circumcised women than non-circumcised women. E.coli was the most prevalent bacteria among circumcised and non- circumcised women.

Keywords: Antimicrobial; Circumcised Women; Resistant Bacteria; Sudan; UTIs.

Urinary tract infections (UTIs) can affect any part of the urinary system, and women are more likely than men to develop UTIs[1]. Generally, UTIs occur when bacteria enter the urinary tract through the urethra and begin to multiply in the bladder. Despite the urinary system's designed defenses, these can sometimes fail. When that happens, bacteria may take hold and grow into a full-blown infection in the urinary tract. Women experience more than one infection during their lifetimes[2]. The anatomy of the female urethra is of particular importance to the pathogenesis of UTIs. The female urethra is relatively short compared to the male urethra, and it is situated close to the warm, moist, per rectal region, which is highly populated by microorganisms[1].

Other risk factors include: sexual activity, certain type of birth control and menopause (after menopause a decline in circulating estrogen causes change in the urinary tract that make women more vulnerable to infection)[2], urinary tract abnormalities (babies born with urinary tract abnormalities), blocking in the urinary tract; as kidney stone, catheterize (people who can't urinate on their own and use a tube to urinate), urinary procedure (which can be recent urinary surgery or an exam of the urinary tract that involves medical instrument)[3]. Female genital mutilation (FGM) also known as female genital cutting or female circumcision that don't allow the urine to leave completely or cause urine to back up in the urethra have an increased risk of UTIs and menstrual problem[4]. At least 200 million girls and women alive today have undergone FGM[7] living in 30 countries in Africa, the Middle East and Asia where FGM is practiced[8]. The most serious type of FGM is type three that create covering seal and small opening is left for urine and menstrual blood to escape[4]. FGM is thought to be a tribal tradition or an Islamic imperative, and several studies have reported the reasons that a girl might undergo FGM include providing her with an honorable social life, preserving her virginity, and allowing her to become a mature woman for a safe marriage[5, 6]

The overall prevalence of urological complications in women with genital mutilation is 20%. Recurrent urinary tract infections, lower urinary tract symptoms, urinary retention, urogenital fistulas, meatus stenosis, urethral stones, and megaurethra are the reported ones[9]. All those risk factors can lead us to recurrent infection and may result in antibiotic resistant bacteria. Also geographic variation in etiologic agents of UTIs and their resistance patterns in antibiotics[10, 11]

UTIs is the second most common infection presenting in community practice which associated with elevated antibiotic resistance in Sudan[12]. Female genital mutilation may be increasing UTIs[5].

To our knowledge, there are few studies concerning the consequences of circumcision on the urinary system and its risk for increasing antibiotic-resistant bacteria associated with UTIs. So, the ultimate goal of this study is to provide information about this problem which may highlight circumcision and its association with antibiotic-resistant bacteria causing UTIs.

METHODS

This cross-sectional, case-control, hospital-based study was conducted in two clinics in Khartoum locality. The study was carried out between July 2019 and March 2021. The study included 80 Sudanese women (n=80) with genital mutilation (n=40 case groups) and 40 non-genital mutilated women (n=40 control groups); all of them had UTI symptoms including (pyuria, loin pain, burning micturition, frequency, urinary retention and urgency of urination); and with different ages.

Ethical considerations

Ethical approval No. (MLS-RB-07-3-18) to conduct this study was obtained from the Scientific Research Committee, Collage of Medical Laboratory Science, Sudan University of Science and Technology and Dan and Nobatia Clinics in Khartoum Locality. Informed consent was obtained from participants before collection of the urine samples.

Data collection

Data were collected using direct interview the patients, which provided information conceding each case examined.

Laboratory processing

Specimen collection and processing

The participants collected mid-stream urine specimens in a sterile wide-mouth container after they were informed of the collection procedure, and all specimens were processed within one hour of collection.

Isolation

Aseptically, and with a 0.005 calibrated loop, a loop full of well-mixed urine was incubated aerobically at 37°C overnight on blood and Cysteine Lactose Electrolyte Deficient (CLED) agar plates.

Identification of the isolated bacteria

Identification of the isolated bacteria was done by colonial morphology after assessment of significant bacteriuria (>10^5 CFU/1ml of urine), Gram's stain and conventional biochemical tests.

Biochemical tests for identification of Grams negative rods

Grams negative rods were identified by conventional Biochemical tests including: (Kligler Iron Agar (KIA), Indole hydrolysis test, Motility test[13]. Citrate utilization test, Oxidase test[14] and Urease test[15].

Biochemical tests for identification Gram positive cocci

All catalase positive Gram positive cocci isolates and suspected to be *Staphylococci* were identified by coagulase test, DNAse test and fermentation on Mannitol salt agar. Suspected *Enterococcus* isolates which were catalase test negative were identified by litmus milk decolonization test and aesculin hydrolysis test[15].

Susceptibility Testing

Modified Kirby-Bauer susceptibility testing technique

By using a sterile wire loop, 3–5 well-isolated, purified and fresh colonies of similar appearance of the tested organism were touched and emulsified in 3–4 ml of sterile physiological saline. The turbidity of the suspension was matched to the standard turbidity (0.5% McFarland standard) on good light. Sterile swab was used to inoculate a plate of Mueller Hinton agar. Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension. Streaked the swab evenly over the surface of the medium in three directions, rotated the plate approximately 60 to ensure even distribution. With the petri dish lid in place, allowed about 3–5minutes (no longer than 15 minutes) for the surface of the agar to dry. By using a sterile forceps, the appropriate antimicrobial discs was placed, evenly distributed on the inoculated plate. After overnight incubation, the plates was examined to ensure the growth was confluent and by a ruler the diameter of each zone of inhibition was measured in mm. The zone sizes of each antimicrobial disc was interpreted by interpretative chart, according to CLSI guidelines[16].

Table 1. Demographic data of the Study population

Marital status	Circumcised women	Non circumcised women
Single	23(57.5%)	33(82.5%)
Married	17(42.5%)	7(17 %)
Total	40(100%)	40(100%)
Age group	Circumcised women	Non circumcised women
0-20yrs	1(2.5%)	16(40%)
21-40yrs	29(72.5%)	22(55%)
41-60yrs	8(20%)	2(5%)
61-80yrs	2(5%)	0(0%)
Total	40(100%)	40(100%)

Table 2. Association between circumcision and UTIs

Growth rate	Study groups		O.R	*P.value*
	Circumcised women	Non-circumcised women		
Growth	34(85%)	6(15%)	32.11	0.001
No growth	6 (15%)	34 (85%)		
Total	40 (100%)	40(100%)		

Table 3. Association Gram's reaction of the isolates and study groups

Isolates	Study groups		Total	*P.* value
	Circumcised women	Non-circumcised women		
G-ve rods	24 (60.0%)	6 (15.0%)	30 (75.5%)	2.35
G+ve cocci	10 (25.0%)	0 (0%)	10 (25.0%)	
Total	34 (85.0%)	6 (15.0%)	40 (100.0%)	

Table 4. Frequency of isolated bacteria among circumcised and non- circumcised women

Isolated bacteria	Study groups		O.R	*P.* value
	Circumcised women	Non- circumcised women		
E.coli	12(35.3%)	3 (50%)	5.11	0.001
K.pneumoniae	7 (20.6%)	1(16.7%)	8.27	
Pr.mirabilis	2 (5.9%)	1(16.7%)	2.05	
Ps.aeruginosa	3 (8.8%)	1(16.7%)	3.16	
S.aureus	1 (2.9%)	0 (0%)	-	
S.saprophyticus	1 (2.9%)	0 (0%)	-	
E.faecalis	8 (23.5%)	0 (0%)	-	
Total	34/40 (100.0%)	6 /40 (100.0%)	-	

Table 5. Antimicrobial susceptibility of isolated bacteria among circumcised women

IsolatesNo. (%)	Antimicrobial susceptibility (Sensitive)					
	Amp	Cip	Gen	NA	Aug	Cef
E.coli (n=12)	5 (41.6%)	4(33%)	7(58%)	0(0%)	62 (50 %)	3 (25%)
K.pneumoniae(n=7)	2 (28%)	4 (57.1%)	3 (42.8%)	0(0%)	6 (85%)	3 (42.8%)
Pr.mirabilis(n=2)	0 (0%)	1 (50 %)	2(100%)	0(0%)	2(100%)	1(50%)
Ps.aeruginosa(n=3)	0 (0%)	2 (66.6%)	1 (33.3%)	0(0%)	1(33.3%)	(0%)
En,faecalis (n=8)	2 (25%)	3/8 (37.5%)	5 (62.5%)	-	5 (62.5%)	3 (37.5%)
S.aureus (n=1)	0 (0%)	1 (100%)	1 (100 %)	-	1 (100%)	1 (100%)
S.saprophyticus(n=1)	1(100%)	1 (100%)	1 (100%)	-	1 (100.0%)	1 (100 %)

Key: Amp=Amoxycillin, Cip= Ciprofloaxacin, Gen= Gentamicin, NA = Nalidixic acid, Aug=Augmentin, and Cef= Cefuroxime

Table 6. Antimicrobial susceptibility of isolated bacteria among non-circumcised patients

Antimicrobial agents Isolates	*E.coli* N0. (%)	*K.pneumoniae* N0. (%)	*Pr.mirabilis* N0. (%)	*Ps.aeruginosa* N0. (%)
Amoxycillin	0 /3 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
Ciprofloxacin	1/3 (33.3%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
Gentamicin	3/3 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
Nalidixic acid	0 /3 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
Augmentin	3/3 (100%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
Cefuroxime	2/3 (66.6)	1/1 (100%)	1/1 (100%)	1/1 (100%)

Statistical analysis

The obtained data from this study was analyzed using statistical package of the social science (SPSS) version 20.0. Frequencies were expressed in form of table and Chi-Square test was used to determine the significant differences at *p*-value d"0.05.

RESULTS

A total of 80 urine specimens had been collected as follow: 40 were circumcised and other 40 were not, and their ages ranged from 7 to 70 years old with a mean age of 28.8± 13.8 S.D. About 16/40 (40%) of circumcised women were married and 23/40 (57%) were single, while among non-circumcised group there were 7/40 (17%) married and 33/40 (82%) single as in table 1. The growth rate was 34/40 (85%) among circumcised females and 6/40 (15%) among non-circumcised participants and there was high statistical difference between circumcision and UTIs (*P*.value=0.001) and circumcised women were 32 times exposed to risk of UTIs than non-circumcised one as showed in table (2). The isolated bacteria were 24/34 (70.6%) G-ve rods and 10/34 (29.4%) G+ve cocci among circumcised women, while all isolated bacteria were G-ve rods 6/6 (100%) and no isolated G+ve cocci among non-circumcised one as displayed in table (3).

Among the isolated bacteria; *Escherichia coli* was the most predominant isolate among circumcised and non-circumcised women. Circumcised women were 5, 8, 2, and 3 times more susceptible to urinary tract infection with *Escherichia coli, K.pneumoniae, Portus mirabilis,* and *Ps*eudomonas *aeruginosa,* respectively than non-circumcised ones. There was a high association between the isolated organism and circumcised women (*P*. value=0.001) as illustrated in table (4). The all isolated bacteria from circumcised women were highly sensitive to Gentamycin (7/12 (58.0%)) compared to other antimicrobial agents and in non-circumcised was 100.0% for Gentamycin and Augmentin. Also all isolated G-ve rods in both groups were totally resistant to Nalidixic acid as displayed in table (5) and (6).

DISCUSSION

In this study, the growth rate was 34 (85%) among circumcised women rather than non-circumcised 6 (15%). This result almost confirms the theory of Elduma when he mentioned UTIs as one of the circumcision complications in Sudan (2018)[5]. In the current study, the circumcised women were more likely to have and develop UTIs (85%) than the non-circumcised group (15%). This result was similar to that obtained by Amin *et al*. (2013)[17] in Egypt which reported highly significant different types of UTIs by 86.6% of the study group (251 circumcised women participate in the study).

Gram negative rods isolated more frequently than G+ve cocci in this study, 24/34 (70.6%) and 10/34 (29.4%), respectively in circumcised women, Also in non-circumcised women Gram negative rods also grew more frequently than G+ve cocci in 6/40 (15%) of the cases, but there were no isolated G+ve cocci among circumcised women. This was harmonized to that reported in Sudan by Saeed who found 45(65%) Gram negative bacteria more than Gram positive 24(35%) from 69 of significant growth urine sample[12].

The present study found the commonest frequent was *E. coli* 12(35.3%), (followed by *En. faecalis* 8(23.5%) and *K. pneumoniae* 7(20.6%) among circumcised women, Moreover, while *E. coli 3*(50%) was the most prevalent among non-circumcised women. It was close to the study carried out by Theodore[18] in Nigeria who found 141 out of 181 urine specimens and *E.coli* was 47 (33.3%) followed by *Klebsiella species* 28 (19.9%) and 5(3%) *En. faecalis*. Also among non-circumcised females *E.coli* was more predominant than others.

The isolated bacteria from circumcised women were highly resistant to a number of the antimicrobial agents used, including: Nalidixic acid (100%), Amoxycillin (70.5%), and cefuroxime (64.7%). This was in agreement with a study conducted by Ahmed and colleagues[19] in Sudan, in which *E.coli* showed high resistance to amoxicillin, but low resistance to nalidixic acid. In non-circumcised women, (83%) were resistant to Nalidixic acid and Amoxycillin, whereas (0%) were resistant to cefuroxime.

According to our knowledge studies on urinary tract infections in women who undergo genital mutilation are lacking in the literature; and no studies have been conducted to compare these results. In general, circumcised women were more resistant to antimicrobial agents than non-circumcised women. One of the limitation of this study; it was conducted on a limited number of samples.

CONCLUSIONS

UTIs and antimicrobial-resistant bacteria were more prevalent among circumcised women than non-circumcised women. *E.coli* was the most prevalent bacteria among circumcised women followed by *En. faecalis* and *K.pneumoniae*.

Recommendations

Increase awareness of the population about the risk of female genital mutilation which is a critical health problem. Additional research studies in a broader field, including different areas in Sudan, and using more advanced methods like polymerase chain reaction (PCR) will be necessary. Prevention or decrease of female genital mutilation, which is still a traditional custom in Sudan.

Conflict of Interests

The authors declare that they have no competing interests.

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REFERENCES

1. Tille PM. Bailey and Scott's diagnostic microbiology, 14th ed. Saint Louis: Elsevier; 2017. p. 987-992.
2. Hertting O. Urinary Tract Infection Pathogenesis and Complications. Department of Microbiology, Tumor and Cell Biology Karolinska Institutet, Stockholm, Sweden: Shannon Wright, .2011. p.1-16.
3. Mayo Clinic (2019). Urinary tract infection. https://www.mayoclinic.org/diseases-conditions/urinary-tract-infection/symptoms-causes/syc-20353447 (Accessed on 14/09/2019, 9.00 p.m.).
4. https://en.wikipedia.org/wiki/Female_genital_mutilation (accessed on 18/9/2019, 8.25 p.m).
5. Elduma AH. Female genital mutilation in Sudan. *Open access Macedonian journal of medical sciences*. 2018 Feb 15;6(2):430.
6. Abathun AD, Sundby J, Gele AA. Attitude toward female genital mutilation among Somali and Harari people, Eastern Ethiopia. *Int J Women's Health.* 2016;8:557
7. Unicef. Female genital mutilation (FGM). August 2021.
8. WHO. Female genital mutilation. 21 January 2022.
9. Zambon JP, Mihai B, Ivan CA, Magalhaes RS, Karakci A, Zhao S. Urological complications in women with genital mutilation. *Clin Med Rep.* 2018;1:1-4.
10. Gupta K. Emerging antibiotic resistance in urinary tract pathogens. *Infect Dis Clin North Am*. 2003 Jun;17(2):243-59
11. Akoachere JF, Yvonne S, Akum NH, Seraphine EN. Etiologic profile and antimicrobial susceptibility of community-acquired urinary tract infection in two Cameroonian towns. *BMC Research notes*. 2012 Dec;5(1):1-8.
12. Saeed A, Hamid SA, Bayoumi M, Shanan S, Alouffi S, Alharbi SA, Alshammari FD, Abd H. Elevated antibiotic resistance of Sudanese urinary tract infection bacteria. *EXCLI journal.* 2017;16:1073.
13. Goldman E, Green LH. The family Enterobacteriaceae. Practical handbook of microbiology, 2nd edn. CRC Press, Taylor & Francis Group, New York. 2009:220-222.
14. Kumar S. Essentials of Microbiology. *JP Medical Ltd;* 2015 Nov 30. P.p. 48-55.
15. Cheesbrough M. District laboratory practice in tropical countries, part 2. Cambridge University press; 2005. Pp.35-70.
16. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing.* (2018) supplement M100, 28th ed. Wayne, PA: 2018.
17. Amin MM, Rasheed S, Salem E. Lower urinary tract symptoms following female genital mutilation. *International Journal of Gynecology & Obstetrics*. 2013 Oct 1;123(1):21-3.
18. Theodore M. Prevalence of Urinary Tract Infections among prison imamates in Nigeria. *The internet Journal of Microbiology*. 2006;3(2):4394.
19. Ahmed AA, Osman H, Mansour AM, Musa HA, Ahmed AB, Karrar Z, Hassan HS. Antimicrobial agent resistance in bacterial isolates from patients with diarrhea and urinary tract infection in the Sudan. *The American journal of tropical medicine and hygiene.* 2000 Nov 1;63(5-6):259-63.