Antimicrobial Sensitivity Patterns Of Urogenital Bacterial Isolates Among Hiv Positive Patients In The Federal Medical Centre In Gombe

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Abstract

Apart from the risk of increasing the concentration of HIV infection in genital secretions, consequences of sexually transmitted infections (STI) can be grave, resulting in conditions such as infertility, ectopic pregnancy, cancer and neonatal infections. Urogenital tract swab samples including urethral swabs (URS), endocervical swabs (ECS) and high vaginal swabs (HVS) were aseptically collected from 158 HIV positive individuals presented with symptoms of urogenital tract infections at federal medical centre, Gombe between May to September, 2009. These were subjected to microscopy, culture and sensitivity testing (using Abtec® sensitivity discs). Out of the samples, 30 (18.9%) produced bacterial growth. In the male category, highest percentage of bacteria isolated was in the age group 11-20yrs (20.0%), while in the female category, it was in the age group 41-50yrs (25.0%). No bacteria isolated at age ≥51yrs in both sexes. Six opportunistic bacterial species were isolated of which Staphylococcus aureus had the highest frequency of 9 (30.0%) while Enterococcus faecalis had the lowest as 1(3.3%). The most active drug against S. aureus observed was augmentin (71.4%). Two exogenous bacterial species were also isolated; Neisseria gonorrhea which was resistant to tetracycline and Haemophilus species which was sensitive to all but cotrimotazole and fusidin. This work recommended the need for the hospitals to improve in partner notification and treatment, early and correct diagnosis to the patients and use of augmentin, gentimicin and sparfloxacin in symptomatic treatment, where laboratory investigations are not available.

INTRODUCTION

Pathogenic bacterial isolates of urogenital tract (UGT) are among sexually transmitted infections (STI) that have a significant probability of transmission between humans or animals by means of sexual contact, especially during vaginal, oral or anal sex. (1).

Urogenital tract bacterial infections may be divided into two main categories; infections due to opportunistic (endogenously acquired) bacteria such as Staphylococcus aureus or, Escherichia coli. These are the most common cause of urogenital tract diseases, (2) and infections due to exogenously acquired bacteria e.g. Treponema pallidum and Neisseria gonorrhea, (3).

Although most of UGT pathogenic bacteria are treatable, however, even the once easily cured have now become resistant to many of the older traditional antibiotics. (4). In antimicrobial therapy of these infections, it is equally clear however that host response are at least as important as are the antibiotics in determining the outcome of an infection.

The recent emergence of HIV/AIDS has provided an ample evidence of the importance of host defence in protecting infection as the high mortality due to AIDS is almost entirely attributed to opportunistic infections, hence the need for a very special management of the opportunistic infections.

The synergistic relationship between STI and HIV is well recognized. In Africa, transmission of the virus takes place mainly through heterosexual intercourse (5). Studies have shown that STI increase concentration of HIV in genital secretions (6) and thus improved clinical management of STI significantly reduces the incidence of HIV infection in developing countries (7). In Nigeria, the median prevalence of HIV among STI patients was found to be 11.5% (8).

Apart from the risk of acquiring HIV infection, consequences of STI can be grave resulting in conditions such as infertility, ectopic pregnancy, cancer, neonatal infections and even death (9). Although STI are common to both sexes and all ages in any community, certain risk

factors abound for acquiring the diseases, e.g. younger age (15-39 years), having more than one sexual partner and irregular use of condoms (10).

MATERIALS AND METHODS

STUDY SITE/GROUP

The study was conducted between May and September, 2009 in Federal Medical Centre Gombe, a tertiary health institution located within the city of Gombe, the capital of Gombe State, Nigeria. Study group involved were patients attending the hospital from within and outside the state.

ETHICAL CONSIDERATION

This work was granted ethical clearance by the ethical committee of federal medical centre Gombe.

SAMPLES COLLECTION

Samples were aseptically collected from one hundred and fifty eight (158) HIV–positive patients with symptoms of urogenital tract infections and registered for the purpose of the study. Among them were 32 urethral swab (URS), 41 endocervical swab (ECS) and 85 high vaginal swab (HVS) samples.

For each sample, sterile hand gloves were put and cotton wool moistened in sterile normal saline (N/S) was used to clean round of the genital opening. For URS sample, the urethra was gently massaged from above downwards (2-3 times). The discharge (or pus from penile ulcer) was then collected directly on a sterile swab stick. For ECS sample, the cervix was cleansed using sterile swab moistened in sterile N/S. Another sterile swab stick was then inserted down to the endocervical canal and gently rotated so as to obtain the specimens. In case of HVS sample, the folded layers of the vagina was cleansed with cotton wool moistened in sterile N/S. High vaginal discharge was then collected using sterile swab stick. All samples were carefully and properly labeled and taken to the laboratory immediately.

BACTERIAL ISOLATION

Specimens were immediately and aseptically inoculated onto 10% Blood agar (BA) and MacConkey agar (MCA) plates, incubated aerobically at 37° C overnight. The specimens were also inoculated on 10% Chocolate agar (CA) plates but incubated in a moist CO_2 enriched atmosphere at 37°C overnight.

IDENTIFICATION

Colonial morphological features, Gram stain microscopy and biochemical tests (catalase, coagulase, oxidase, citrate, motility, indole, urea, glucose/lactose utilization, gas and hydrogen sulphide production) were carried out according to standard procedures as stated by Cheesbrough, (11).

CHARACTERIZATION

The isolates were characterized based on the Bergey's manual for determination of bacteria species as shown by Don, (12).

ANTIBACTERIAL SUSCEPTIBILITY TESTS

Four to five well isolated colonies of a confirmed pathogen were picked with a sterile wire loop and inoculated on Mueller-Hinton agar, 10% Blood agar or Chocolate agar (depending on the organisms) according to standard disc diffusion method (13). The antimicrobial agents used were augmentin (30µg/ml), cloxacillin (5µg/ml), cotrimoxazole (25µg/ml), erythromycin (5µg/ml), fusidic acid (20µg/ml), gentamicin (10µg/ml), sparfloxacin (10 µg/ml) and tetracycline (10 µg/ml), prepared as antibiotic sensitivity discs by Abtek, (13). The plates were immediately incubated at 37°C overnight. The diameters of the zones of inhibitions were measured to the nearest whole millimeters, (14).

RESULTS

Table 1 shows the occurrence of bacteria isolated from the three different urogenital sites with age. Among the URS category highest percentage prevalence of bacterial isolates was observed in the age range 11- 20, representing 20.0%, in the ECS category it was at age range 41-50 with 33.3% and in the HVS category it was at age range 21-30 with 22.7%.

Table 2 had summarized the frequency of the bacterial isolates in HIV infection. In the males category, 32 patients were tested and 4 (12.5%) yielded co-infections. In the females' side, 126 patients were tested with 26 (20.6%) co-infections. In general, out of the 158 HIV-positive patients, 30 (18.9%) had bacterial co-infection. In the female category, there was increase in percentage bacterial isolates with increase in age from 18.2% (11-20); 20.6% (20-31); 23.1% (31-40); and 25.0% (41-50). No bacterial isolate was observed at age ≥51yrs in both sexes.

Table 3 had shown the 8 different bacterial species isolated in the HIV-positive patients as co-infections. Among the opportunistic infections (OIs), Staphylococcus aureus had the highest frequency of 9 (30.0%) while Enterococcus

faecalis had the lowest frequency observed as 1(3.3%). Only 2 exogenous bacterial infections were isolated and they include; Neisseria gonorrhea: 2 (6.6%) and Hemophillus species 1 (3.3%).

T he antimicrobial susceptibility pattern of the isolates was summarized in Table 4. The most active drug against S. aureus was augmentin (71.4%), while against E. coli was gentamycin (80.0%) and against Klebsiella pneumonia was sparfloxacin (75.0%). The Neisseria gonorrhea isolated was resistant to tetracycline while Haemophilus sp was sensitive to all but cotrimotazole and fusidin. In general, gentamycin, sparfloxacin and augmentin were the most active drugs observed.

Figure 1

Table 1: Bacterial isolates from urogenital swab samples among HIV-positive patients

Age range	Urogenital swab samples						Total	
	Urethral swab		Endocervical swab		High vaginal swab			
	NT	BI (%)	NT	BI (%)	NT	BI (%)	TT	TBI (%)
11-20	5	1 (20.0)	6	1(16.7)	16	3(18.8)	27	5 (18.5)
21-30	11	1 (9.1)	24	4 (16.7)	44	10 (22.7)	79	15 (18.9)
31-40	15	2 (13.3)	8	2 (25.0)	18	4 (22.2)	41	8 (19.5)
41-50	1	0 (0.0)	3	1 (33.3)	5	1 (20.0)	9	2 (22.2)
≥51	0	0 (0.0)	0	0 (0.0)	2	0 (0.0)	2	0 (00.0)
Total	**32**	**4 (12.5)**	**41**	**8 (19.5)**	**85**	**18 (21.2)**	**158**	**30 (18.9)**

Key: NT= Number Tested, BI= Bacteria Isolated, TT= Total Test, TBI= Total Bacteria Isolated

Figure 2

Table2: Bacterial isolates across age and sex of HIV-positive patients

Age range	Sex				Total	
	Male		Female			
	NT	BI (%)	NT	BI (%)	TT	TBI (%)
11-20	5	1(20.0)	22	4 (18.2)	27	5 (18.5)
21-30	11	1 (9.1)	68	14 (20.6)	79	15 (18.9)
31-40	15	2 (13.3)	26	6 (23.1)	41	8 (19.5)
41-50	1	0 (0.00)	8	2 (25.0)	9	2 (22.2)
≥51	0	0 (0.00)	2	0 (0.00)	2	0 (0.00)
Total	**32**	**4 (12.5)**	**126**	**26 (20.6)**	**158**	**30 (18.9)**

Key: NT= Number Tested, BI= Bacteria Isolated, TT= Total Test, TBI= Total Bacteria Isolated

Figure 3

Table 3: Bacterial species identified among HIV-positive patients

Bacteria isolated	Number isolated (%)
Staphylococcus aureus	9 (30.0)
Escherichia coli	7 (23.3)
Klebsciella pneumoniae	5 (16.5)
Pseudomonas aeruginosa	3 (10.0)
Entrobacter species	2 (6.6)
Neisseria gonorrhea	2 (6.6)
Hemophillus species	1 (3.3)
Entrococcus faecalis	1 (3.3)
Total isolates (8)	**30 (18.9)**

Figure 4

Table 4: Antibiogram sensitivity pattern of bacterial isolates among HIV-positive patients

Bacterial isolates	NBI	Antibiotic activity (%)							
		Aug	Clox	Cot	Ery	Fus	Gen	Spar	Tet
S. aureus	9	77.8	22.2	0.0	44.4	22.2	11.1	22.2	22.2
Escherichia. coli	7	14.3	0.0	28.6	0.0	14.3	85.7	71.4	14.3
K. pneumonia	5	20.0	20.0	20.0	20.0	20.0	40.0	80.0	20.0
Ps. aeroginosa	3	66.7	0.0	0.0	0.0	0.0	100	66.7	0.0
Enterococcus sp.	2	50.0	50.0	50.0	50.0	100	100	50.0	50.0
N. gonorrhea	2	100	50.0	50.0	100	100	100	100	0.0
H. ducreyi	1	100	100	0.0	100	0.0	100	100	100
E. faecalis	1	100	0.0	0.0	0.0	0.0	100	100	100

Key: NBI: Number of Bacteria Isolated, Aug: Augmentin, Clox: Cloxacillin, Cot: Co-trimoxazole, Ery: Erythromycin, Fus: Fusidic acid, Gen: Gentamycin, Spar: Sparfloxacin, Tet: Tetracycline.

DISCUSSION

In this work relatively high bacterial isolates were obtained from both ECS; 8 (19.5%) and HVS; 18 (21.2%) as against URS; 4 (12.5%). This may be related to the anatomical feature of the female urethra as Allen and Ronald, (15) suggested that high incidence of urogenital bacterial infections among women can be attributed to the short anatomical feature of the female urethra which favors easy access and penetration by pathogens.

T otal bacterial isolates observed from HIV patients in this study was 4(12.5%) in the male category and 26 (20.6%) in the female category. These were high enough to suggest that bacterial infection of the UGT may increases the risk of HIV

transmission or disease progression, as Sagar, (16) mentioned that urogenital bacterial infections can cause transmission or increase in HIV disease progression in two major ways. One, they could cause the disruption of the normal epithelial barrier by genital ulceration and/or microulceration as they could also cause increase in the accumulation of pools of HIV-susceptible or HIV-infected cells in semen and vaginal secretions.

It was also reported by Myer, (17) that women could become more susceptible to HIV-1 infection with increase in age because of hormonal changes, increased vaginal microbial ecology and changes in physiology. This might likely be the reason why, in female category, there was increase in percentage bacterial isolates with increase in age from 18.2% (11-20); 20.6% (20-31); 23.1% (31-40); and 25.0% (41-50). No bacterial isolate was observed at age ≥51yrs in both sexes. This might possibly be as a result of long term medication prior to sample collection as suggested by Keith, (18).

A mong the opportunistic bacterial infections isolated in this study, Staphylococcus aureus had the highest percentage prevalence of 9 (30.0%). This was close to 31.5% similarly obtained by Olowu and Oyetunji, (19). Such higher occurrence of Staphylococcus aureus could be attributed to its minimal growth requirements, high resistance to environmental factors and ability to colonize and establish infection in almost every site of the body, in addition to decrease in immunity of the patients as suggested by Akelere, (20).

The isolates next to S. aureus in prevalence were two members of Enterobacteriaceae: Escherichia coli: 7 (23.3%) and Klebsciella pneumoniae: 5 (16.5%). This finding was supported by, Shitu and Mandara, (21) who in a study carried out at Zaria reported E. coli as the most prevalent isolate among women attending antenatal clinics at Zaria. While Olowu and Oyetunji, (19) recorded Klebsiella species as the most prevalent isolate with 47.5%. This finding also agreed with report by Hooton, (22) that bacteria from gastro intestinal tract (fecal flora) are the most common cause of gastro intestinal tract diseases among sexually active women. This is possible because of the short distance between the two sites. Secondly colonization of the vaginal introitus and periurethral area, by these bacteria may lead to ascending of the urethra to the renal pelvis and parenchyma for them to initiate renal infection. Johnson, (23) also added that, the frequent incidence of the members of Enterobacteriaceae family, such as E. coli and Klebsiella species is possible because they normally produce virulence factors (adhesins, K-antigens and hemolysins) which enhance their colonization of the vaginal cells, thus making attachment and invasion very easy.

T he isolation of 2 (6.6%) Neisseria gonorrhea is in line with what had been obtained in other parts of Nigeria such as 4.0% in Enugu (24) and 3.0% in Potiskum (25). To buttress this findings a report according to CDC, (26), had shown that gonoccocal urethritis account for up to 80% of acute urethritis cases in certain underdeveloped regions of the world and that it is equally much more common in men than in women. Wilson and Sande, (1) also seconded that whenever pelvic inflammatory disease (PID) developed as a complication of cervicitis, N. gonorrhea and C. trachomatis are the pathogens primarily responsible.

The main pathological feature of HIV infection is the progressive destruction of the immune system, which is commonly observed when there is involvement of other opportunistic infections, (27). In this study, Pseudomonas aeruginosa infection in HIV patients was observed to be resistance to the antibiotics employed with the exception of gentamicin, augmentin and sparfloxacin. This may be possible due to reasons mentioned by Adome, (25) that fairly costly drugs like augmentin are hard to be abuse in Northern Nigeria (possibly due to poverty), sparfloxacin being a new generation of fluoroquinolones drugs may not be well known to drug abusers while gentamicin which is only administered intra muscularly, may not easily be abused by patients.

A s many strains of H .ducreyi produce B-lactamase, and therefore making it is possible for antibiotic that is B-lactamase stable or one that inhibits its production to have a high activity against the isolates (28), however the susceptibility of Hemophillus species to all antibiotics used in this study with exception of co-trimoxazole and fusidin may not be surprised as the two drugs are easily used by drug abusers. While co-trimoxazole is very cheap, orally administered and it is also used for prophylactic treatment of opportunistic infections in HIV patients, fusidin on the other hand is manufactured as tablets, gel cream, ointment or suspension which can all be abused easily.

In conclusion, the findings from this study had shown that urogenital bacterial infections have significant effect in transmission and diseases progression in HIV patients and that prevalence of bacterial infection among them was found

to be relatively higher in females than in males. The most active drugs against urogenital bacterial isolates in HIV patients, as observed from this work, were sparfloxacin, gentamicin, and augmentin.

O n the ground of the above conclusions, we wish to recommend that, more efforts should be placed in moral development of our youth through involvement of community and religious leaders. Hospitals should provide professional counseling, encourage partner notification and treatment while laboratories should insist on early and correct diagnosis in addition to use of standard antibiotic sensitivity discs. Where investigations are not available gentamicin, augmentin, and sparfloxacin antibiotics could be included in syndromic treatment of urogenital tract diseases, especially in Gombe state and its environment as high activity of these drugs were observed from this study.

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