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Full Length Research Paper

Prevalence of multi-drug resistant pathogen isolated from high vaginal swab in Nigeria

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Samples of 262 high vaginal swabs collected from patients attending Ogun State College of Health Technology, Ilese-Ijebu were screened for sexually transmitted diseases (STDs) pathogen and antimicrobial susceptibility pattern. Micro-organisms isolated from these samples include; *Niesseria gonorrhea, Staphylococcus aureus, Gardnerella vaginalis, Candida albican* and *Escherichia coli*. Age group 21 to 25 had the highest occurrence of pathogens. About 81.8% of the pathogens isolated showed resistance to five or more antibiotics while 6.81% showed resistance to one antibiotic. *S. aureus* was found to be the most sensitive when tested against Ofloxacine and Nitrofuratoin with 25 zone of inhibition. *G. vaginalis* had minimum inhibitory concentration (MIC) ranging from 32 to 128 µg/ml while *N. gonorrhea* had MIC ranging from 16 to 512 µg/ml when tested a gainst tetracycline, ampiclause, ampicilline, gentamycin and augmentin. *G. vaginalis* had minimum bactericidal concentration (MBC) ranging from 32 to 128 µglml fo r tetracycline, nitrofuratoin, ampicilline, ampiclause and metronidazole while *N. gonorrhea* had MBC of 32 µg/ml for tetracycline and 512 µg/ml for ampicilline, ampiclause and gentamycin. Since micro-organisms continue to develop resistant to antibiotics, constant antimicrobial surveillance required to provide safety and effective therapy.

Key words: Antibiotics, STD pathogens, multi-drug resistance, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

INTRODUCTION

Sexually transmitted diseases (STDs) can be defined as infection spread from person to person through sexual contact, exchange of semen, blood and other body fluids or by direct contact with the affected body areas of people with sexually transmitted diseases (Chinsembu, 2009).

Sexually transmitted are caused by bacteria infection, virus, fungi, protozoan and metazoan. This sexual contact can involve vaginal, oral and anal sex. Some STDs can also be passed to another through other means such as blood transfusion or from an infected mother to her baby during pregnancy or child birth (Boschert, 2009).

STDs are very common, especially among young people ages 15 to 24. There are about 19 million new cases in the world each year (Janet et al., 2002). STDs are highly preventable if diagnosed early such as gonorrhea and Chlamydia can be easily treated and cured before serious complications develop. Other such as HIV/AIDS and genital herpes are not curable, but prompt diagnosis and treatment can help to reduce or delay the onset of serious complication, improve quality of life and minimize the spread of the disease to others (Janet et al., 2002).

The center for diseases control and prevention (CDC) has reported that 85% of the most prevalent infectious diseases in the United States are sexually transmitted

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(CDC, 1998). About 12 million new STD infections occur in the United States each year (CDC, 1998). One in four occurs in someone between the ages of 16 and 19 (CDC, 1998). Almost 65% of all STDs infections affect people under the age of 25 (Chinsembu, 2009). Most of these organisms especially bacteria are now becoming resistant to most of the antibiotics commonly used in the treatment of STDs. Therefore, this paper investigated the prevalence of culture-able STD bacteria that are multidrug resistant.

MATERIALS AND METHODS

Samples collection

High vaginal swab was obtained from two hundred and sixty two patients attending Ogun State College of Health Technology Clinic Ilese Ijebu with chronic complains of vaginal discharge, itching and irritation with or without excessive mal odorous discharge. The samples were collected using sterile swabs stick and speculum.

Isolation of microorganisms

Isolation of the microorganisms was done by inoculating the collected samples on Blood, Chocolate and Sabouraud agar. Blood and Sabouraud agar plates were incubated aerobically while Chocolate agar plates were incubated micro-aerophilically at 37°C for 48 to 72 h (Osoba, 1979). Microaerophilic incubation was in a candle jar supplied appropriately 5 to 10% carbon dioxide.
Wet preparations were equally done for the presence of clue cells, yeast cells and so on. The isolates were identified using morphological appearance and biochemical tests.

Culture preservation

The pure cultures of the isolates were sub-cultured into maintenance medium. It was then incubated at 37°C, the stock culture were stored at 4°C for subsequent use.

Characterization of isolates

Characterization of isolates was carried out by employing macroscopic, microscopic and biochemical tests.

Identification of the isolates

The results obtained from the test carried out were used to phenotypically identify the organisms by making reference to Bagey's Manual of Systemic Identification (Sneath, 1986).

Antibiotic susceptibility test

Antibiotic susceptibility test for each bacteria pathogen was performed using the disc diffusion method. 0.1 ml actively growing culture containing 1 × 106 cfu/ml of each bacterium pathogen used was introduced into Petri dishes and 20 ml of molten agar added. The antibiotic sensitivity discs (Abtek Biological Ltd) consisting of different antibiotics namely Augmentin (30 µg), Gentamycin (10 µg), ofloxacin (5 µg), Tetracycline (10 µg), Metronidazole (10 µg), Ampiclause (10 µg), Ampicilline (10 µg), Nalidixic (30 µg), Cotrimoxazole (25 µg), Amoxyline (30 µg), Nitrofuratoin (300 µg), Ceflazidine (30 µg) and Cefuroxime (30 µg) were placed on the solidified agar surface. The plates were incubated aerobically at 37°C for 24 h. After this period, the diameter of the zone of inhibition of each disc was measured. The zone of inhibition corresponded to the antibiotic activity of each disc (Norrby, 1992). Resistance was defined by the absence of a zone of inhibition. The relative susceptibility of each isolate to each antibiotic was shown by a clear zone of inhibition.

MIC-MBC tube technique

Doubling dilution of 256 µg/ml of antibiotics solution was made in 1 ml volume of broth to 0.125 µg/ml. One row of the test was inoculated with 0.02 ml of 1 in 100 dilution of the overnight broth culture of the test organism (Stokes and Ridgway, 1980). The test was incubated at 37°C for 24 h. The minimum inhibito ry concentration (MIC) is the lowest concentration of antibiotics that prevent the visible growth of bacteria after 24 h incubation (Osoba, 1979). At the end of the incubation period, the content of the tube that showed no turbidity was sub-cultured onto Chocolate agar plate for *N. gonorrhoea* and Nutrient agar (for other pathogens) plate for minimum bactericidal concentration (MBC) determination. MBC is the minimum concentration of the antibiotic that is able to kill the microorganism on solid plate completely.

RESULTS

A total sample of 262 high vaginal swabs was collected from patient attending Ogun State College of Health Technology Clinic Ilese Ijebu with complaint of itching and malodorous discharge. The isolates were differentiated using different parameter such as cultural and cellular morphology after which they were subjected to various biochemical studies. From their various biochemical results, they were identified as *Niesseria gonorrhea*, *Gardnerella vaginalis*, *Candida albican*, *Staphylococcus aureus* and *Escherichia coli*.

Number and percentage occurrence of microorganisms isolated from high vaginal swabs of patients attending Ogun State College of Health Technology, Ilese Ijebu (OSCOHTECH) is shown in Table 1. Age group 21 to 25 had the highest occurrence of all the isolates with 8.7% of *S. aureus*, 31.3% of *C. albican*, 3.1% of *E. coli*, 0.76% of *G. vaginalis* and 0.38% of *N. gonorrhea* while the age range 46 to 50 had the lowest of all the isolates.

Antibiotics sensitivity testing of the sexually transmitted pathogens is shown in Table 2. Various antibiotics such as Augmentin, Tetracycline, Ofloxacine, Gentamycine, Nalidixic, Cotrimoxazole, Ceftazidine, Cefuroxime and so on were used. *S. aureus* was found to be the most sensitive organism when tested against ofloxacine and nitrofuratoin with 25 mm zone of inhibition while *G. vaginalis* had the least zone of inhibition when tested against tetracycline and metronidazole with 5 mm and

Table 1. Number and percentage occurrence of microorganisms isolated from HVS of patients attending OSCOHTECH clinic.

Age range	No of samples collected from each age group	Patient positive for *S. aureus* (%)	Patient positive for *C. albican* (%)	Patient positive for *E. coli* (%)	Patient positive for *G. vaginalis* (%)	Patient positive for *N. gonorrhea* (%)
15–20	50	19 (7.25)	26 (9.9)	05 (1.9)	01 (0..38)	Nil
21–25	120	23 (8.7)	82 (31.3)	09 (3.1)	02 (0.76)	0.1 (0.38)
26–30	25	09 (3.4)	15 (5.7)	03 (1.1)	Nil	Nil
31–35	27	06 (2.2)	18 (6.8)	01 (0.38)	01 (0.38)	01 (0.38)
36–40	20	05 (1.9)	12 (4.5)	03 (1.1)	Nil	01 (0.38)
41–45	12	Nil	12 (4.5)	Nil	Nil	Nil
46–50	8	03 (1.1)	05 (1.9)	Nil	Nil	Nil
Total	262	65 (24.81)	170 (64.88)	21 (8.02)	04 (1.52)	03 (1.14)

Table 2. Antibiotic sensitivity pattern of microorganism isolated from HVS of patient attending STDs clinic of Ogun State of Health Technology Ilese Ijebu.

Bacterial isolates	Augmentine(30µg)	Tetracycline(10µg)	Ofloxacine(5µg)	Gentamycin(10µg)	Nalidixic (10µg)	Cotrimoxazole (25µg)	Amoxycline (30 µg)	Ampricilline(10µg)	Nitrofuratola(300 µg)	Metronidazole (10 µg)	Ceftazidine(30µg)	Cefunzime(30 µg)	Apiclause(10µg)
S. aureus	S (15±0.10 mm)	R	S (25±0.15 mm)	S (18±0.18 mm)	R	R	R	R	S (25±0.10 mm)	R	R	R	R
C. albican	NOT			Determined				R	R	R	R	R	R
G. vaginalis	R	S (5±0.01 mm)	R	R	R	R	R	S (10±0.15 mm)	R	S (8±0.12 mm)	R	R	R
E. coli	R	R	S (17±0.14 mm)	S (22±0.12 mm)	S (20±0.16 mm)	R	R	R	S (21±0.15 mm)	R	R	R	R
N. gonorrhea	R	S (15±0.05 mm)	R	S (12±0.02 mm)	R	R	R	S (10+0.13 mm)	R	R	R	R	R

Value are the means ± stand-deviation and R = resistant S= sensitivity (mm) zone of inhibition.

8 mm, respectively.

STDs pathogens were 100% resistant to Ceftazidine, Cefuroxime and Ampiclause. However they were 80% resistant to Metronidazole, Amoxyline and Cotrimoxazole. *N. gonorrhea* was noted to be resistant to almost all antibiotics except tetracycline, gentamycine and ampiciline. Similar result was obtained for *G. vaginalis*. Sensitivity pattern of *C. albican* was not determined being a fungus (Table 3).

About 81.8% of the pathogens isolated from infected STD patients were multidrug resistant; that is they showed resistance to five or more antibiotic while 6.81% of the STD pathogens were resistant to one antibiotic used in this work (Table 4).

Table 5 shows the MIC of antibiotic that is able to inhibit the growth of the pathogen in the liquid medium. *G. vaginalis* had MIC of 32 µg/ml against Nitrofuration while *N. gonorrhea* had MIC of 32 to 256 against Tetracycline, Ampiciline, Ampiclause and Gentamycine. However, *S. aureus* had MIC of 32 to 256 against Augmentin, Gentamycine and Ampiciline.

The MBC was equally determined for the STDs pathogens. MBC of *C. albican* was not determined being a fungus. *G. vaginalis* had MBC ranging

Table 3. Percentage antibiotic susceptibility pattern of clinical STDs isolates.

Antibiotic µg/l	% Sensitivity	% Resistance	% of organisms not determined
Augmentine	20	60	20
Tetracycline	40	40	20
Ofloxacine	40	40	20
Gentamycine	60	20	20
Nalidixic	20	60	20
Cotrimoxazole	0	80	20
Amoxyline	0	80	20
Ampiciline	40	60	0
Nitrofuratoin	40	60	0
Metronidazole	20	80	0
Ceflazidine	0	100	0
Cefuroxime	0	100	0
Ampiclause	0	100	0

Table 4. Number of STD pathogens showing multi-drug resistance.

No of antibiotic	No of Strains showing resistance (%)
One Antibiotic	3(6.81)
2	2(4.5)
3	2(4.5)
4	1(2.27)
5	3(6.81)
6	4(9.09)
7	4(9.09)
8	3(6.81)
9	3(6.81)
10	4(9.09)
11	5(11.36)
12	5(11.36)
13	5(11.36)

from 128 to 512 µg/ml for Nitrofuration, Tetracycline, Ampiciline, Metronidazole and Ampiclause. *N. gonorrhea* had MBC of 32 µg/ml for tetracycline, 512 µg/ml for Ampiciline, Ampiclause and Gentamycine while *E. coli* had MBC of 2 µg/ml against Ofloxacine and Gentamycine; 256 µg/ml against Nalidixic and Ampiciline (Table 6).

DISCUSSION

Two hundred and sixty two samples of high vaginal swab of infected female students attending Ogun State College of Health Technology Clinic Ilese Ijebu were collected and analyzed for pathogenic bacteria. The isolated microorganisms were identified as *S. aureus*, *C. albican*, *E. coli*, *G. vaginalis* and *N. gonorrhea* on the basis of morphological and biochemical parameter which actually agrees with Berge's Manual of Systematic Bacteriology (Sneath, 1986).

Age range 21 to 25 had the highest occurrence of *C. albican*. This may be as a result of the fact that they are sexually active age group. This is in agreement with the study of Janet et al. (2002) that STDs are very common especially among young people ages 15 to 24 and that there are about 19 million new cases in the world yearly.

Most of the pathogens isolated in this study were multidrug resistant to antibiotics. Antibiotic resistance

Table 5. Minimum inhibitory concentration (µg/ml) of micro-organisms isolated from HVS Patients attending STDS clinics.

Bacterial isolates	Nitrofuraloin	Tetracycline	Ampiciline	Ampiclause	Ofloxaciline	Metronidazole	Augmentine	Gentamycin	Nalidixic acid
S. aureus	N.D	ND	256	N.D	N.D	N.D	32	64	N.D
Candida albican	N.D	ND	ND	N.D	N.D	N.D	N.D	N.D	N.D
E. coli	N.D	ND	256	N.D	1	N.D	N.D	2	128
G. vaginalis	32	64	64	64	N.D	64	N.D	N.D	N.D
N. gonorrhoea	N.D	32	256	256	N.D	N.D	N.D	256	N.D

ND – Not done HVS – High vaginal swab.

Table 6. Minimum bacteriocidal concentration (µg/ml) of Micro-organisms Isolated from HVS of Patients attending STDS clinics.

Bacterial isolates	Nitrofuraloin	Tetracycline	Ampiciline	Ampiclause	Ofloxaciine	Metronidazole	Augmentine	Gentamycin	Nalidixic acid
S. aureus	N.D	ND	512	N.D	ND	N.D	32	128	N.D
Candida albican	N.D	ND	ND	N.D	N.D	N.D	N.D	N.D	N.D
E. coli	N.D	ND	256	ND	2	N.D	N.D	2	256
G. vaginalis	128	128	128	512	N.D	128	N.D	N.D	N.D
N. gonorrhoea	N.D	32	512	512	N.D	N.D	N.D	512	N.D

ND – Not done. HVS – High vaginal swab.

could be due to a gene transferred between bacteria in a horizontal fashion by conjugation, transduction or by transformation. Therefore, a gene for antibiotic resistance which had evolved from natural selection may be due to many antibiotic resistance genes reside on Plasmid that facilitate their transfer (Aubry-Damon and Courvalin, 2003). Antibiotic resistance could occur through spontaneous mutation (Ochei and Kolhathar, 2000). Antibiotic resistance also occurs when bacteria continue to proliferate at a rapeutically achievable concentration (Roland, 1984).

In view of this, micro-organisms used; were seen to be resistance to a lot of antibiotics. *G. vaginalis* was resistant to all antibiotics except tetracycline, ampicilline and metronidazole. *S. aureus* also exhibited varying degree of resistance to all except Augmentin, ofloxacine and Gentamycine. If a bacterium carries several resistance genes, it is described as multi resistance or informally, a superbug hence microorganism isolated in this work such as *E. coli*, *N. gonorrhea, G. vaginalis* and *S. aureus* could be described as multi resistant.

Wise and Cornelis (1998) found out that 20 to 50% antibiotics used in human and 40 to 80% in animals is un-necessary and highly questionable. In Denmark for example while 24 kg of active vancomycin was used for human therapy in 1994, 24000 kg of active aroparcin (vancomycin equivalence in veterinary practice) was used as feed additives for animals (Wegener, 1998). In Austria between 1992 and 1996 an annual average of 582 kg of vancomycin was imported for medical purposes and 62, 242 kg of aroparcin for animal husbandry (Witte, 1988). As expected vancomycin resistant *Enterococcus faecium* of animal origin has been detected in human (Komolafe et al., 2003) through consumption of contaminated meat (Bates et al., 1994; Wegener et al., 1997; Schouten and Libdblad, 1997; Wegener et al., 1999; Klare et al., 1999) thus making the treatment of these infection difficult. The prevalence of resistance in this study shows that the resistance profile of the above named STDs pathogen was pronounced for the drugs such as metronidazole, ceftazadine, tetracycline

and ampiclause. The proportion of resistance of pathogens was remarkable. This is similar with what was observed by Lamikanra and Okeke. (1997). The data obtained in this work confirms the indiscriminate use of antibiotics and having more than one sexual partnered are highly prevalent among students and other developing countries (Hart and Kariuki, 1998; Okeke et al., 1999). About 95% of all the antibiotics used in this study showed a considerable rise in resistance, they are commonly used in Nigeria and other developing countries (Hart and Kariuki, 1998). Such multi drug resistance has serious implication for the empiric therapy of infections caused by STDs pathogens.

Almost all the antibiotics used in this study are in-expensive and are widely used even without prescription from authorized health institution; oral consumption of these drugs are known to provide selective pressure ultimately leading to a higher level of resistant bacteria (Levin et al., 1997). This was clearly demonstrated by the percentage resistant bacteria. Those bacteria which their sensitivity was not done were due to the turbidity shown in MIC medium which directly indicates resistance. This work is also in close range with what was observed by Aibinu et al. (2004) who reported 100% resistance of *E. coli*, *C. albican* and *G. vaginalis*.

Since there is continuous development of resistance to the commonly use antibiotics, urgent intervention to provide an alternative therapy such as use of plant extracts and probiotics without antibiotics resistance that will help in prompt treatment of STDs infection is highly needed. Government through Federal Ministry of Health should form a formidable team to monitor the sales and use of antibiotics; there should be a law guiding the use and sales of these drugs in developing countries.

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