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Full Length Research Paper

Multi-drug resistant *Escherichia coli* from Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) patients in Keffi, Nigeria

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Diarrhea is one of the major opportunistic infections associated with human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), with the major causative agent being *Escherichia coli*. This study investigates the occurrence of multi-drug resistance in *Escherichia coli* from patients with HIV/AIDS attending the Federal Medical Center, Keffi, Nigeria. *E. coli* from stool samples of consenting HIV/AIDS patients were isolated and tested for their susceptibility to commonly used antimicrobial agents using the disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI). *E. coli* was isolated at the rate of 76.7%. All the isolates were resistant to antibiotics with lower frequencies for gentamicin (56.1%) and streptomycin (56.8%). The most common resistance phenotypes were SXT, CH, SP, CIP, AM, AU, CN, PEF, S, OFX and SXT, CH, SP, CIP, AM, AU, PEF, OFX with frequencies at 26.5% and 27.3% respectively. All the *E. coli* are multiple antibiotic resistance (MAR) isolates with most (66, 50%) being jointly resistant to eight antibiotics. MAR indices were also very high (all above 0.2) and suggest prior exposure of all the isolates to antibiotics. The high isolation rate provides justification for the monitoring of HIV/AIDS patients for *E. coli*; and testing for susceptibility to antibiotics before prescription will ensure adequate treatment of infection arising from these stool pathogens and reduction in the spread of bacterial resistant strain.

Key Words: Multi-drug resistance, *Escherichia coli*, HIV/AIDS

INTRODUCTION

Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) is a global health problem with an estimated 39.4 million persons (adults and children below 15 years) living with the virus, 4.94 million new infections and 3.11 million deaths (UNAIDS, 2009). The adult prevalence and deaths in Nigeria are respectively 2.5% (corresponding to 3.6 million persons living with the virus) and 310, 000 (UNAIDS, 2010). Due to the depressed immunity that accompanies the infection, HIV/AIDS is often associated with so many opportunistic infections. Multi-organ involvement by opportunistic infections and neoplasms is the major cause of morbidity and mortality in people living with HIV/AIDS (Echejoh et al., 2006).

Opportunistic infections in HIV/AIDS are diverse with diarrhea, due mainly to *Escherichia coli*, as one of the major opportunistic infections (Todar, 2008). Hence, *E. coli* isolated from stool of HIV positive patients should not be ignored, particularly in Nigeria which has a high number of AIDS related deaths, the second highest in the World, after South Africa (Garcia-Jardon et al., 2010; UNAIDS, 2008). It is against this background that this study is undertaken to investigate the isolation frequency and antimicrobial resistance in *E. coli* isolates in HIV/AIDS patients which attend HIV/AIDS Clinic at the Federal Medical Center, Keffi, Nigeria.

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Table 1: Isolation Rate of *Escherichia coli* from HIV/AIDS patients who attend Federal Medical Center, Keffi, Nigeria

No. of stool specimen screened	No. Positive	Isolation Rate (%)
172	132	76.7

Table 2: Rate of resistance to common antimicrobial agents of *Escherichia coli* isolates from HIV/AIDS patients who attend Federal Medical Center, Keffi, Nigeria

Antimicrobial agents	Disc Content (µg)	Number of isolates resistant	Rate of Resistance (%)
Sulfamethoxazole/Trimethoprim (SXT)	30	119	90.2
Chloramphenicol (CH)	30	119	90.2
Sparfloxacin (SP)	10	130	98.5
Ciprofloxacin (CPX)	10	130	98.5
Amoxicillin (AM)	30	129	97.7
Gentamicin (CN)	10	74	56.1
Ofloxacin (OFX)	10	131	99.2
Streptomycin (S)	10	75	56.8
Pefloxacin (PEF)	30	112	84.8
Amoxicillin/Clavulanic acid (AU)	30	131	99.2

MATERIALS AND METHODS

Patients and Ethical Considerations

The study population includes all the HIV/AIDS patients of different age, sex, socio-economic status and tribes receiving antiretroviral treatment (ART) at the Federal Medical Centre, Keffi, Nasarawa State in Nigeria. The study was done between June and October 2010 following receipt of written informed consent of patients and ethical clearance (from the Ethical Committee of the hospital) in line with standard practice.

Processing of samples and culture of isolates

Stool samples were collected in batches using sterile universal containers that were opened only at the point of collection and transported to the laboratory for analysis. The samples were first cultured on MacConkey agar (MAC: Sigma-Aldrich Chemie GmbH, Germany) to isolate lactose fermenters (pink or red colonies). Pink colonies were then transferred to eosin methylene blue agar (EMB: BIOTEC Laboratories Ltd, Ipswich, UK) to differentiate *E. coli* (by its metallic sheen growth on EMB) from other lactose fermenters. Suspected *E. coli* colonies were further confirmed biochemically by a panel of test called "IMViC"- Indole, Methyl Red, Voges-Proskauer and Citrate. Pink or red colonies that grow with metallic sheen on EMB and were indole-positive, methyl red-positive, Voges-Proskauer negative and citrate negative were taken as *E. coli*. Isolates were either used immediately or maintained on slants of nutrient agar (NA: Merck KGaA, Darmstadt, Germany) at 4ºC for future use. All chemicals used were from BDH Laboratory Supplies, Poole, England.

Antimicrobial Susceptibility Testing

Susceptibility testing of all isolates was carried out by disk diffusion technique in accordance with CLSI criteria (CLSI, 2007) using multi-antibiotic discs (Abidec Company, England) containing the following antimicrobials and disc content (in µg): amoxicillin (30 µg), spafloxacin (10 µg), gentamicin (10 µg), pefloxacin (30 µg), chloramphenicol (30 µg), streptomycin (10 µg), ciprofloxacin (10 µg), sulfamethoxazole/trimethoprim (30 µg), ofloxacin (10 µg) and amoxicillin/clavulanic acid (30 µg). *Escherichia coli* ATCC 25922 was used as an internal control. Interpretation of data was based on the standard CLSI chart as updated.

RESULTS

Isolation rate of *Escherichia coli*

The frequency of isolation of *E. coli* from the patients is 76.7% as given in Table 1.

Antimicrobial Resistance

All the isolates were resistant to antibiotics. The rate of resistance of the organisms to different antimicrobial agents is as shown in Table 2. The highest (99.2%) resistance was obtained to amoxicillin/clavulanic acid and

Table 3: Distribution into various resistance phenotypes of antibiotic resistant *Escherichia coli* isolates from HIV/AIDS patients who attend Federal Medical Center, Keffi, Nigeria

Resistance Phenotypes	Number of isolates with the pattern	Frequency (%)
SXT, CH, SP, CIP, AM, AU, CN, PEF, S, OFX	35	26.5
SXT, CH, SP, CIP, AM, AU, CN, PEF, OFX	10	7.6
SXT, CH, SP, CIP, AM, AU, PEF, S, OFX	10	7.6
SXT, CH, SP, CIP, AM, CN, PEF, S, OFX	1	0.8
SXT, CH, SP, CIP, AM, AU, CN, OFX	1	0.8
SXT, CH, SP, CIP, AM, AU, PEF, OFX	36	27.3
SXT, CH, SP, CIP, AM, AU, CN, S, OFX	2	1.5
SXT, CH, SP, CIP, AM, AU, S, OFX	7	5.3
SXT, CH, SP, AM, AU, CN, PEF, OFX	2	1.5
SXT, CH, CIP, AM, AU, CN, PEF, S, OFX	2	1.5
SXT, CH, CIP, AM, AU, CN, S, OFX	1	0.8
SXT, CH, CIP, AM, AU, PEF, S, OFX	1	0.8
SXT, SP, CIP, AM, AU, CN, PEF, S, OFX	3	2.3
SXT, SP, CIP, AM, AU, CN, PEF, OFX	6	4.5
SXT, SP, CIP, AM, AU, PEF, S, OFX	1	0.8
SXT, SP, CIP, AM, AU, PEF, OFX	1	0.8
SXT, SP, CIP, AU, PEF, S	1	0.8
CH, SP, CIP, AM, AU, CN, PEF, S, OFX	1	0.8
CH, SP, CIP, AM, AU, CN, S, OFX	9	6.8
CH, SP, CIP, AU, CN, PEF, S, OFX	2	1.5

SXT = Sulfamethoxazole-Trimethoprim; CH = Chloramphenicol; SP = Sparfloxacin; CIP = Ciprofloxacin; AM = Amoxicillin; AU = Amoxicillin-Clavulanic acid; CN = Gentamicin; PEF = Pefloxacin; S = Streptomycin; OFX = Ofloxacin

Table 4: Frequency of multiple antibiotic resistances and multiple antibiotic resistance indices of *Escherichia coli* isolates from HIV/AIDS patients who attend Federal Medical Center, Keffi, Nigeria

Number of antimicrobial agents isolates are resistant to	Number of isolates with MAR	Frequency (%)	*MAR indices
6	1	0.8	0.6
7	1	0.8	0.7
8	66	50.0	0.8
9	29	22.0	0.9
10	35	26.5	1.0

*MAR index = No. of antimicrobial agents isolate is resistant to/No. of antimicrobial agents tested (that is, 10)

ofloxacin; while the least was to the aminoglycoside antibiotics gentamicin (56.1%) and streptomycin (56.8%), making these aminoglycosides the most acceptable for use to treat *E. coli* infections in these patients.

Antibiotic Resistance Phenotypes

The distribution of the isolates into different antibiotic resistance phenotypes observed is as given in Table 3. The most common resistance phenotypes are SXT, CH, SP, CIP, AM, AU, CN, PEF, S, OFX and SXT, CH, SP, CIP, AM, AU, PEF, OFX with frequencies of (26.5%) and (27.3%) respectively.

Multiple antibiotic resistance (MAR) and MAR indices

Multiple antibiotic resistance, defined here as joint resistant to more than two antimicrobial agents, is present in all the isolates, with most (66 of them) being jointly resistant to eight (8) antimicrobial agents. The frequency of MAR is as given in Table 4. MAR indices above 0.2 indicate that such isolates originate from environment where antimicrobial agents are freely available and accessible with high potential for abuse (Krumpermann, 1983).

DISCUSSION

The high isolation rate of *E. coli* could be as a result of the compromised (depressed) immunity associated with HIV/AIDS which make them prone to infection (Echejoh et al., 2006).

The relatively lower resistance of the isolates to the aminoglycoside antibiotics (gentamicin and streptomycin) could be attributed to their requirement for parenteral administration which hinder their misuse and abuse due the discomfort associated with injections. The widespread

and inappropriate use of antibiotics is a significant contributing factor to the development and spread of bacterial resistance to antimicrobial agents (Mincey and Parkulo, 2001). For most bacteria, there is evidence that increased usage of a particular antimicrobial correlates with increased levels of bacterial resistance (Granizo et al., 2000).

Resistance to antibiotics by *E. coli* is not new (Ngwai et al., 2005; Xiao et al., 2005; Sanders and Sanders, 1992). The observation that some isolates were resistant to streptomycin but not to gentamicin could be explained by the fact that gentamicin, in addition to binding to a specific S12 protein in the 30S ribosome, also binds to the L6 protein of the 50S ribosome to inhibit protein synthesis (Tripathi, 2003). Hence, a possible alteration of the S12 protein target alone in the streptomycin-resistant isolates is incapable of affecting its action.

The high frequency of multiple antibiotic resistances observed is probable indication of abuse and misuse of antibiotics in this environment. This is supported by the high MAR indices observed. An MAR index (a tool that reveals the spread of bacterial resistance in a given population) above 0.2 implies that the strains of such bacteria originate from an environment where several antibiotics are used (Krumpermann, 1983).

In conclusion, monitoring HIV/AIDS patients for *E. coli* during each visit is recommended. The prior exposure of isolates to antimicrobial agents as suggested by their high MAR indices provides justification for continuous monitoring of bacterial susceptibility to antibiotics before prescription in order to ensure adequate treatment of infections arising from stool pathogens and reduction in the spread of bacterial resistant strain. The emergence and spread of antimicrobial resistance is an important public health issue.

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