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Effect of Trimethoprim-Sulfamethoxazole and Azithromycin Prophylaxis on Antimicrobial Resistance of Faecal *Escherichia coli* Isolated from HIV-Infected and TB Patients in Ekiti State

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Abstract

Aim: To determine the effect of Trimethoprim-Sulfamethoxazole and Azithromycin Prophylaxis on Antimicrobial Resistance of Faecal *Escherichia coli* isolated from HIV-Infected and TB Patients in Ekiti, Nigeria.

Method: A total of 360 samples were collected of which 300 isolates of *E. coli* were obtained from the stool samples of HIV/AIDS patients on treatment, HIV/AIDS patients not on treatment, HIV/TB co-infected patients, TB patients on treatment, TB patients not on treatment and apparently healthy individuals. The sample was cultured on Eosin-Methylene Blue (EMB) agar plate and incubated at 37°C overnight. Colony showing the greenish metallic sheen was Gram stained, single rod shaped (Bacilli), motile, indole positive, Simmons citrate negative, yellow butt and yellow slant with gas production on Triple Sugar Ion Agar (TSIA) were considered to be *E. coli.*

Result: A total of 141 (47%) males and 159 (53%) female patients were involved in the study. Based on the age distribution, age group 30 to 39 (n=80) has highest percentage while age group 60 and above (29) has the lowest participation among the age groups. *E. coli* isolated from HIV/TB co-infected reveals 40 (80%) and 41 (82%) resistant to SXT and AZM respectively while 23 (46%) and 29 (58%) of *E. coli* isolated from HIV patients on treatment were susceptible to SXT and AZM. Tuberculosis patients on anti-TB treatment had 42 (84%) and 40 (80%) of the isolates resistant to SXT and AZM respectively while *E. coli* isolated from newly diagnosed HIV patients were 25 (50%) and 12 (24%) of the *E. coli* were resistant to SXT and AZM. Similarly, 28 (56%) and 11 (22%) of the isolated *E. coli* from newly diagnosed TB patients show resistance to SXT and AZM respectively.

Conclusion: Faecal *E. coli*, an indicator organism for enteric pathogens, rapidly develops resistance to SXT and AZM which are clinically important antimicrobials after their initiation as prophylaxis.

Keyword: *E. coli*; HIV/AIDS; TB; Trimethoprim-Sulfamethoxazole; Azithromycin

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Introduction

There were approximately 1.2 million HIV positive new TB cases globally in 2014 [1]. About 74% of these people live in developing and under developed regions like Africa [2]. The highest increase in tuberculosis cases has occurred in locations and groups with the largest HIV prevalence rate which implies that the HIV infection is at least partially responsible for the increase of tuberculosis [3]. Evidence has shown that immune responses in tuberculosis and other infection produce encourage the replication of HIV which give rise to full blown AIDS in patients [4,5]. At least one-third of the 37 million people living with HIV worldwide have latent TB. Summarily, people living with HIV have more chances (24% to 28%) or are more likely to develop active tuberculosis than those without HIV [2,6,7]. However, as a result of the impaired immunity that accompanies the HIV infection, HIV/AIDS is often associated with so many opportunistic infections with Tuberculosis (TB) being the highest commonly presented case among HIV patient, including those already on antiretroviral therapy [8]. The risk of TB in HIV-infected patients and the impact of TB diagnosis on disease progression in HIV-infected patients have been well documented in many Africa countries [9,10]. TB and HIV/AIDS can be worsened by each other. TB is the most common opportunistic

Table 1: Socio-demographic associates of SXT resistivity amongst subjects under study.

Variable	SXT Resistivity				
	Yes (%)	No (%)	χ^2	p-value	AOR (95% CI)
Age Group (in years)					
<30	38 (50.7)	37 (49.3)	12.026	**0.017**	1
30-39	49(61.2)	31 (38.8)			1.382 (0.721-2.649)
40-49	51 (69.9)	22 (30.1)			1.955 (0.979-3.906)
50-59	24 (55.8)	19 (30.1)			1.144 (0.534-2.449)
60 and above	24 (82.8)	5 (17.2)			4.275 (1.464-12.482)
Gender					
Male	88 (63.3)	51 (36.7)	0.188	0.664	-
Female	98 (60.9)	63 (39.1)			-
Marital Status					
Single	50 (55.6)	40 (44.4)	2.445	0.485	-
Married	113 (65.3)	60 (34.7)			-
Separated	9 (60.0)	6 (40.0)			-
Widowed	14 (63.6)	8 (36.4)			-
Education					
Primary	19 (61.3)	12 (38.7)	1.153	0.562	-
Secondary	44 (67.7)	21 (32.3)			-
Tertiary	123 (60.3)	81 (39.7)			-
Occupation					
Student	35 (56.5)	27 (43.5)	5.742	0.057	-
Civil Servant	68 (56.7)	52 (43.3)			-
Business	83 (70.3)	35 (29.7)			-
Residence					
Urban	106 (56.7)	81 (43.8)	5.954	0.015	1
Rural	80 (70.8)	33 (29.2)			1.658 (0.988-2.781)

AOR-Adjusted Odd Ratio from the binary logistic regression analysis for the socio-demographic predictors of SXT resistivity 95% CI to 95% Confidence Interval of the AOR

disease and cause of the death for those infected with HIV [11]. Similarly, HIV infection is one of the most important risk factors associated with an increased risk of latent TB infection progressing to active TB disease [12,13]. So the WHO's Policy on collaborative TB/HIV activities recommends a combination of measures to reduce the burden of TB among HIV-infected individuals [14]. These measures include intensified case finding, isoniazid preventive therapy, and infection control and antiretroviral therapy. However, TB may occur at any stage of HIV infection [15]. The management of TB infections in persons with HIV has been improved through the use of antibiotics prophylaxis such as Trimethoprim-Sulfamethoxazole and Azithromycin respectively. Serious bacterial infections pose a major threat to HIV and TB patients, especially when immunity is impaired. Antimicrobial prophylaxis with Trimethoprim-Sulfamethoxazole (SXT) and Azithromycin (AZM) has been used for more than a decade for the prevention of opportunistic infection among HIV and TB patients. Thus, SXT and AZM have been used in hundreds of thousands of patients worldwide particularly in African countries [16,17].

Materials and Methods

A total of 360 samples were collected from HIV/AIDS, Tuberculosis (TB), HIV/AIDS/TB co-infected patients on standard treatment regimen and normal individual without any underline disease or infection attending clinics at Tertiary Hospitals in Ekiti State. To determine whether there were changes in Sulfamethoxazole and Azithromycin (AZM) sensitivity, Sensitivity study was done using *E. coli* strains from stool sample collected from new TB cases, TB patients on standard Anti-TB treatment which include Sulfamethoxazole or Azithromycin, New HIV cases, HIV patients on treatment regimen Using Sulfamethoxazole or Azithromycin as prophylaxis and TB/HIV negative individuals who has not taken Sulfamethoxazole or Azithromycin.

Culturing and identification of colonies

The stool sample was initially cultured on MacConkey agar (OXOID) plate and incubated at 37°C overnight. Suspected *E. coli* strains were sub cultured and purity and identification on EMB agar plate. Representative colonies of the bacterial that showed the characteristic black metallic chin coloration were selected; Gram stained and identified through strings of conventional biochemical reactions.

Antibiotics susceptibility

Antibiotics susceptibility testing of the isolates was done using Kirby-Bauer method on Mueller-Hinton agar according to CLSI, 2015. Inoculated plates were incubated overnight at 37°C for 18 hours to 24 hours, after which antibiotics sensitivity of the *E. coli* were tested

Table 2: Socio-demographic associates of Azithromycin Resistance amongst subjects under study.

Variable	AZM Resistivity				
	Yes (%)	No (%)	χ^2	p-value	AOR (95% CI)
Age Group (in years)					
<30	32 (42.7))	43 (57.3)	22.36	<**0.001**	1
30-39	56 (70.0)	24 (30.0)			**3.86 (1.92-7.81)**
40-49	54 (74.0)	19 (26.0)			**3.82 (1.80-8.15)**
50-59	31 (72.1)	12 (27.9)			**3.47 (1.44-8.47)**
60 and above	22 (75.9)	7 (24.1)			
Gender					
Male	89 (64.0)	50 (36.0)	0.107	0.743	-
Female	106 (65.8)	55 (34.2)			-
Marital Status					
Single	49 (54.4)	41 (45.6)	7.874	**0.049**	1
Married	117 (67.6)	56 (32.4)			**1.75 (1.00-3.05)**
Separated	12 (80.0)	3 (20.0)			3.35 (0.80-16.13)
Widowed	17 (77.3)	5 (22.7)			2.84 (0.88-9.72)
Education					
Primary	18 (58.1)	13 (41.9)	1.01	0.603	-
Secondary	41 (63.1)	24 (36.9)			-
Tertiary	136 (66.7)	68 (33.3)			-
Occupation					
Student	34 (54.8)	28 (45.2)	6.365	**0.041**	1
Civil Servant	75 (62.5)	45 (37.5)			1.37 (0.70-2.68)
Business	86 (72.9)	32 (27.1)			**2.21 (1.11-4.44)**
Residence					
Urban	112 (59.9)	75 (40.1)	5.691	**0.017**	1
Rural	83 (73.5)	30 (26.5)			**1.85 (1.08-3.19)**

AOR-Adjusted Odd Ratio from the binary logistic regression analysis for the socio-demographic predictors of AZM resistivity 95% CI to 95% Confidence Interval of the AOR

using AZM=Azithromycin (15 µg), SXT=Sulfamethoxazole (25 µg).

Statistical analysis

Data obtain from the research was analyzed statistically using Anova and chi square of the SPSS (Statistical Procedure for Social Science).

Result

A total of 360 stool samples were obtained from interested patients and individuals among which 40 samples were rejected and 20 samples did not yield any growth. 300 isolates of *E. coli* were obtained from the stool samples of diarrhoeic and non-diarrhoeic HIV/AIDS patients on treatment (n=50), HIV/AIDS patients not on treatment (n=50), HIV/TB Co-Infected patients (n=50), TB patients on treatment (n=50), TB patients not on treatment (n=50) and individuals (n=50) (non-reactive HIV and smear negative TB) who were attending clinics at major tertiary Hospitals in Ekiti State, Nigeria. The source distribution revealed a total of 141 (47%) males and 159 (53%) female patients were involved in the study. Source distribution of the isolates based on the age of the patients with highest percentage participation among the group of 30 to 39 (n=80) followed by the ≥ 30 group. *E. coli* isolated from HIV/TB co-infected reveals 40 (80%) and 41 (82%) resistant to SXT and AZM respectively while 23 (46%) and 29 (58%) of *E. coli* isolated from HIV patients on treatment were susceptible to SXT and AZM. Tuberculosis patients on anti-TB treatment had 42 (84%) and 40 (80%) of the isolates resistant to SXT and AZM respectively while *E. coli* isolated from newly diagnosed HIV patients were 25 (50%) and 12 (24%) of the *E. coli* were resistant to SXT and AZM. Similarly, 28 (56%) and 11 (22%) of the isolated *E. coli* from newly diagnosed TB patients show resistance to SXT and AZM respectively.

Discussion

The socio-demographic data revealed that the age of patients from where the isolates under study were obtained has a significant association with their susceptibility to the antibiotics tested (SXT, AZM). *Escherichia coli* isolated from patients within the Ages of 30 to 39, 40 to 49 and 50 to 59 and 60 years and above are more statistically significantly resistant than the isolates from subjects with less than 30 years of age (Table 1-3). This is in agreement with finding of Sahuquillo-Arce et al. [18] who indicated that increasing age is associated with resistance McGregor et al. [19] and who reported that resistance is due to increase consumption of antibiotics by older age groups. Marital status and occupation was not found to have any serious relationship with SXT which was contrary to the pattern seen in AZM where the two factors (Marital status & Occupation) were significantly associated with the resistance which are in accordance with the works of Nwadike et al. and Olakolu et al. [20]. The effect of

Table 3: Differences in resistance patterns of AZM and SXT between the different categories of patients and the controls.

Variable	Category of Subject					
	TB/HIV* (%) n=50	HIV* (%) n=50	TB* (%) n=50	HIV (%) n=50	TB (%) n=50	Control (%) n=50
SXT						
Resistant	40 (80.0)	23 (46.0)	42 (84.0)	25 (50.0)	28 (56.0)	11 (22.0)
Intermediate	8 (16.0)	24 (48.0)	6 (12.0)	21 (42.0)	4 (8.0)	10 (20.0)
Sensitive	2 (4.0)	3 (6.0)	2 (4.0)	4 (8.0)	18 (36.0)	29 (58.0)
	χ^2=134.512, p-value**<0.001**					
AZM						
Resistant	41 (82.0)	29 (58.0)	40 (80.0)	12 (24.0)	11 (22.0)	8 (16.0)
Intermediate	8 (16.0)	12 (24.0)	8 (16.0)	2 (4.0)	9(18.0)	6 (12.0)
Sensitive	1 (2.0)	9 (18.0)	2 (4.0)	36 (72.0)	30 (60.0)	36 (72.0)
	χ^2=83.771, p-value**<0.001**					

*Patients on drugs

Bold p-values showed significant differences at 0.05 level.

living environment (Rural and Urban area of residence) on antibiotic resistance appears to be coming to the lime light as a serious topic of discussion with respect to susceptibility of a specific antibiotic. The mechanism behind this is unclear, but this effect has also been reported by Nomamiukor et al. [21]. There are statistically significant differences between the susceptibility of isolates from the urban and rural areas as well as the susceptibility of the isolates from the different age groups. Although there is evidence that increasing antibiotic consumption may be related to increase per capital income [22,23], the varied degree of resistance to the antibiotics exhibited by the isolates with respect to location and age could be related to the income inequality and antibiotic consumption as indicated by Kirby and Herbert [24], the density of general practitioners as indicated by Masiero et al. [25] and educational level, environmental exposure, economic stress and access to personal support as indicated by Ilic et al. [26] and Mangrio et al. [27]. Another possible explanation is the erroneous use of these antibiotics in a population and in veterinary medicine over a period of time increases an individual's probability of contacting an already-resistant organism, such that the potential for susceptibility to the selection of antibiotics within an individual is reduced [18,21]. Direct exposure to antibiotic therapy is believed to be a major factor in resistance to antibiotics [28,29]. It was also observed by Seidman et al. [30] that AZM resistance in *E. coli* is increasing significantly which does not agree with the work of Morpeth et al. [31] that suggested the use of Azithromycin (AZM) in place of Trimethoprim-Sulfamethoxazole (SXT) as prophylaxis against opportunistic infections among HIV infected patients. Equally, Rogers et al. [32] in their studies revealed that resistant isolates at a previous sampling time has been found to have a higher odds of subsequent resistance carriage. We noted that prior antibiotics exposure and resistance status would be an important predictor of current resistance status. It was suggested that once resistance was established within an individual, it would be sustained which is in agreement with Rogers et al. [32]. Although Lay et al. [33] believed that Diarrheagenic *E. coli* were approximately twice as likely to be resistant to SXT compared to non-pathogenic isolates from the same faecal specimen [34]. It is therefore a limitation of our study that we could not differentiate and compare the resistance between different *E. coli* pathotypes.

Conclusion

In conclusion, this work reveals that faecal *E. coli*, an indicator organism for enteric pathogens, rapidly develops resistance to SXT and AZM which are clinically important antimicrobials after their initiation as prophylaxis. These data suggest that while the substantial benefits of SXT and AZM prophylaxis are realized in Africa, the resistance of these antibiotics to *E. coli* is on the increase and needed a serious attention. Long-term studies are needed to evaluate the impact of use of SXT and AZM prophylaxis in Africa on other factor relating to HIV and TB patients. HIV/AIDS and TB patient's treatment regimen was not observe to have a significant effect on the isolation of *E. coli* from stool samples but the study reveals that the HIV/AIDS and TB patient's treatment regimen may affect the susceptibility of *E. coli* to SXT and AZM. Significant resistance was seen among *E. coli* isolated from HIV and TB patients, supporting that commensal *E. coli* can serve as an indicator organism for circulation of pathogenic strains.

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