

Drug Resistance Pattern of Diarrheogenic *Enterobacteriaceae* among HIV Seropositive Individuals on Cotrimoxazole Prophylaxis at St. Paul's Hospital, Addis Ababa

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Abstract

Back ground: Reduced efficacy of available drugs and anti-microbial resistance are challenges for control of infectious diseases. In recent years multi-drug resistant strains of bacteria has posed major public health problems. Hence prevention and control strategies should be implemented based on the studies available on pattern of drug resistance and prevalence of infectious agents particularly in resource limited countries, furthermore periodic surveillance of antibiotic resistance and effectiveness against common bacterial infections should be monitored.

Objectives: To assess antibiotic resistance pattern of diarrheogenic *Enterobacteriaceae* among HIV infected ART-Naïve as well as ART-experienced individuals.

Methods: A hospital based cross-sectional study was conducted on individuals taking co-trimoxazole prophylaxis and presented with complaints of diarrhea. Stool samples were collected and examined by culture and sensitivity testing was done to evaluate antibiotic resistance patterns of selected diarrheogenic *Enterobacteriaceae*.

Result: From a total 162 HIV sero-positive co-trimoxazole taking patients presented with diarrhea, three types of bacterial isolates were observed in 14.8% of study participants. Most Prevalent bacterial isolates were *Shigella species* (6.8%), *Salmonella paratyphi* (4.9%) and *Salmonella typhi* (3.1%). Most frequently recovered bacterial isolates were resistant to co-trimoxazole with over all resistance pattern of (91.7%) and this isolates were also susceptible to Ceftraxone; next to Ceftraxone, the isolates was sensitive to Chloramphenicol (66.7%) than Nalidixic acid and Nitroforantione. The most multi drug resistant bacteria isolates were *S.typhi* which is 100 resistant against co-cotrimoxazole, Ampicilline, Nitroforantine and Nalidixic acid. Despite high resistance pattern of co-trimoxazole, statistically significant improvement of CD4 T cell level was seen after cotrimoxazol prophylaxis and highly active anti-retroviral treatment, compared to baseline count with the mean difference of 169/mm³ CD4 T-cell (paired t-test, p<0.001). WHO clinical stage, types of water source and raw vegetable consumption did not show a significant association with enteric bacterial infection and antibiotic resistance.

Conclusion: In present study, bacterial resistance to co-trimoxazole was found to be very high; hence, its use for routine treatment of acute diarrheal illness is questionable.

Key words: Co-trimoxazole, Diarrheogenic *Enterobacteriaceae*.

Introduction

According to recent estimate, 9 countries have more than 14% prevalence of HIV and 1.7 million HIV related deaths worldwide. Among this reported cases, most commonly infected were individuals at productive age thus results unprecedented burden on the economic growth of sub-Saharan countries (UNAIDS 2012). After introduction of ART and series of effort made to reduce the burden and incidence of the diseases, progressive decline of incidence and improvement of lives of cases with HIV/AIDS were reported in most countries: Contrary to the decline of HIV prevalence in some countries, new HIV infections continue to outpace those added on antiretroviral treatment. Particularly in sub-Saharan Africa where the prevalence is highest, increased death rate among midlife adults was evident in association with the fueling factor for HIV death such as co infection with TB and other Opportunistic infections (OIs) (Edith et al. 2008). The condition also aggravated by immune

suppuration as a result of low CD4 count. Worldwide there are between 12 and 18 million HIV infected individuals with CD₄ cell counts of 200 cell/ micro liter or less those encountered one or more opportunistic related infectious diseases. The majority of these OIs were bacterial infections and intestinal parasites which cause life-threatening acute or chronic watery diarrhea (UNAIDS 2005). Pathogens such as *Salmonella* and non-typhoid *Salmonella*, which typically only causes enteritis in healthy individuals, can lead to severe septicemia in immune compromised patients (Jacobson et al. 1989). Episodes of bacterial enteritis in HIV infected patients with diarrhea, also contribute for mortality in HIV infected individuals (Nadelman 1985).

Reduced efficacy of available drugs and anti-microbial resistance is becoming challenges for control and prevention of infectious diseases in

immune compromised hosts; in recent years the emergence and global dissemination of multi-drug

resistant typhoidal strains of *Salmonella* has also posed major public health problems in developing countries. The condition is more aggravated by overuse, misuse, misdiagnosis and use of selected antibiotics for multiple infectious agents (Threlfall & Stoberingh 2000). The amplifying effect of antimicrobial therapy on resistance is also apparent with the transfer of resistance plasmids to previously susceptible strains (Kenneth J & Rayan C 2004). Due to its proven effect on OIs, co-trimoxazole (800mg SMZ + 160mg TMP) prophylaxis was been recommended for high risk group of patients (WHO/UNAIDS 2000) (Carlucci et al. 2008). It is provided routinely after emergence of AIDS pandemic in most developed world following the promising findings of two studies in West Africa (Anglaret et al. 1999). On the other hand wide spread use of co-trimoxazole antimicrobial agent as prophylactic drug has shown negative impact on the control of microbial resistance. Extended use would promote corresponding population-level resistance with resultant loss of the utility of TMP-SMZ to treat life-threatening bacterial infections (Mermin et al. 2004). Daily TMP-SMZ may reduce its susceptibility to commensal intestinal *Escherichia coli*, increasing the burden of TMP-SMZ-resistant pathogens. Data shows prevalence of TMP- SMZ resistance ranged from (71%) to (81%) after 2 weeks use of this drug as a prophylaxis. It is also evident from similar findings, TMP-SMZ-resistant *E. coli* increased significantly from 78% to 98% among persons taking daily TMP-SMZ (Chiller et al. 2009).

Bacterial diseases and drug resistance control strategies should be implemented based on the studies on the pattern of drug resistance in a given categories of patients with certain disease condition; furthermore, periodic surveillance of antibiotic resistance and effectiveness of available antibiotics against common bacterial infections should be monitored. In Ethiopia, studies in the area of microbial drug resistance are much more limited, even most studies didn't consider the prevalence after intervention with some kind of chemoprophylaxis like co-trimoxazole (TMP-SMZ) and isoniazid (INH) in association with different risk factors. Therefore the present study was conducted to see drug resistance pattern of selected diarrheogenic *Enterobacteriaceae* among co-trimoxazole prophylaxis's taking HIV infected ART-Naïve as well as ART-experienced individuals on ART taking co-trimoxazole prophylaxis at St. Paul's Hospital Millennium Medical College ART Clinic.

Materials and Methods

Study design: A cross-sectional hospital-based study was done at St. Paul's Hospital Millennium Medical

College from December 1, 2013 to March 31, 2014 after approved by the study area IRB. Written consent was obtained from each participant and laboratory results were communicated with the physicians in charge.

Sample collection, inoculation and identification:

Stool samples were collected and inoculated into labeled enrichment culture medias. The standard bacteriological culture methodology was followed for stool culture, which involved inoculating a sample into selective enrichment broth medium and sub culturing in to differential and selective agar media followed by incubated at 37°C for 18 to 24 hours. The sample inoculums were read based on the colony characteristic chart. Biochemical tests were done to confirm *Salmonella spp.* as per bacteriology technical manual ((Monica C 2000).

Antimicrobial susceptibility test: Susceptibility tests were performed using standard Modified Kirby-Bauer Disc Diffusion susceptibility testing technique against the following antimicrobial agents: Chloramphenicol, Trimethoprim/Sulfamethoxazole, Ciprofloxacin, Ceftriaxone, Nalidixic acid, Nitroforantion, and Ampicillin. The test was performed on Mueller-Hinton agar medium. The turbidity of nutrient broth was standardized equivalent to 0.5 McFarland standards (Monica C 2000). The diameter of the zone of inhibition around the disc was measured to the nearest millimeter, inhibition zone diameters for each organism fall within the limits of the standard. The interpretation of the findings was done by referring to the table of critical diameters classified as sensitive and resistant according to National Committee for Clinical Laboratory Standard (NCCLS 2005). Quality of Bacteriological analysis checked by Control strains to check functionality of different media, incubating conditions and biochemical media with bacterial strain of *E. faecalis* ATCC 29212/33186.

Data analysis: The data obtained from this study was analyzed using SPSS version 18.0 Software. Data were summarized using frequency tables and level of resistance summarized in percentages for each isolated bacteria. Multivariate analysis was calculated for associated factor. Odds of bacterial isolates and chi-square or student T test were used. P-value of < 0.05 was considered significant within 95% CI.

Results

Demographic Characteristic: From the total 162 patients participated in the study, 158 (97.5%) of them were from Addis Ababa. Ninety seven percent of the study participants fall between the age group of 26-45 years old and the median age were 36 years old (Table 1). From total study participants, 120 (74.1%)

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were on co-trimoxazole (800mgSMZ+ 160mg TMP)
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treatment

(HAART) and females comprised 78 (48.1%). The remaining 42(25.9%) were on pre ART chronic follow up care with 32(19.7%) female. Seventy six percent of study participants were under Co-trimoxazole prophylaxis for six and more. Fifteen percent of study participants were smear positive TB patients. Around 10.4%, 7.4% and 3% of study participants were on INH, both on INH and HAART, and INH and co-trimoxazole prophylaxes respectively.

BaselineCD₄, current CD₄ and risk factor: About sixty percent of study participants base line CD4 count were below 200/mm³ with a mean CD4 count of 175 cell/mm³ (Table 2). After HAART and co-trimoxazole prophylaxis, significant improvement of CD4-T cell level was seen compared to baseline count with the mean difference of 169/mm³ CD4 T-cell (paired t-test, p<0.001).

Characteristic	CD4 T-cell value(mm ³)	frequency	Percentage
Baseline CD4	<100	45	27.8
	101-200	52	32.1
	201-250	23	14.2
	251-350	40	24.7
	>350	2	1.2
Current CD4	<100	14	8.6
	101-200	24	14.8
	201-250	15	9.3
	251-350	37	22.8
	>350	72	44.4
Total		162	100.0

Table 1: Age and Sex distribution of study participants, St .Paul's Millennium medical college, in 2014

Characteristic		Total	Frequency (%)
Sex	Male	52	(32.0)
	Female	110	(67.9)
Age	18-25	15	(9.3)
	26-35	58	(35.8)
	36-45	58	(35.8)
	46-55	20	(12.3)
	56-65	8	(4.9)
	>66	3	(1.9)
Total		162	100

Table 2: Baseline and current CD4 T-cell counts, St. Paul's Millennium medical college, 2014

Majority (97.5%) of study participants had access for toilets and piped water supply. Nearly half (48.8%) and 44.4% of them had regular habits of drinking

treated water with chlorinating agent and raw vegetables consumption respectively.

Bacterial isolates and resistance pattern: Of 162 stool culture and drug sensitivity testing, 24(14.8%) diarrhea-causing *Enterobacteriaceae* were isolated excluding *E.coli*. The prevalent isolates were *Shigella* spp, *Salmonella paratyphi* and *Salmonella typhi* respectively (Table 3). Antimicrobial drug sensitivity of isolates showed high level of resistance to the majority of the drugs tested. Most bacterial isolates

were resistance against co-trimoxazole with 87.5% minimum resistance pattern observed for *S.paratyphi* and 100% resistance pattern for *S. typhi*. The overall resistance patterns of the isolates against this antimicrobial agent were 91.7%. Next to Co-trimoxazole, *S. paratyphi* and *S. typhi* isolates were 100% resistance against Ampicillin (Table 4).

Table 3: Prevalence of diarrheogenic *Enterobacteriaceae*, St. Paul's Millennium medical college, in 2014

Bacterial isolates	Frequency	Percent
No growth	139	85.8
<i>S. paratyphi</i> .	8	4.9
<i>S. typhi</i> .	5	3.1
<i>Shigella</i> spp.	10	6.2
Total	162	100.0

Ceftriaxone was sensitive for *Shigella* spp. and *S. para typhi* with the sensitivity of 90.9% and 62.5% respectively and showed overall resistance pattern of only 20.8%. Next to ceftriaxone, Ciprofloxacin and Chloramphenicol were sensitive for *Shigella* spp with the same cumulative resistance patterns of 33.3%. *S.typhi* was found to be the most multi drug resistance isolates with 100% resistant to Co-trimoxazole, Ampicillin, Nitroforantine and Nalidixic acid. In addition, it showed 80% resistant to

Chloramphenicol. Isolates of *S.paratyphi* also shows 100% resistance to Ampicillin and Nalidixic acid. *Shigella species* isolates were susceptible for most antimicrobial agent with 100% to Chloramphenicol (Table 4). Regarding the risk factors assessed, toilet use habits, type of water source, raw vegetable consumption and WHO clinical stage has no significant association with wide resistance pattern of the isolates.

Table 4: Antibigram patterns of some diarrheogenic *Enterobacteriaceae* isolates, St. Paul's Hospital Millennium medical college, in 2014.

Antibiotic& potency used/ µg concentration	Sensitivity Pattern	Organismisolated							
		<i>S.paratyphi</i>		<i>shigella</i> spp.		<i>S.typhi</i>		Total	
		No	%	No	%	No	%	R%	
Trimethoprim-1.25	Resistance	7	87.5	10	90.9	5	100.	91.7	
Sulfamethoxazole23.75	Sensitive	1	12.5	1	9.1	0	0.0		
Ampicillin(10 µg)	Resistance	8	100.0	9	81.8	5	100.0	91.7	
	Sensitive	0	0.0	2	18.2	0	0.0		
Chloramphenicol (30 µg	Resistance	4	50.0	0	0.0	4	80.0	33.3	
	Sensitive	4	50.0	11	100.0	1	20.0		
Nitroforantion(30 µg	Resistance	5	62.5	3	27.3	5	100.0	54.2	
	Sensitive	3	37.5	8	72.7	0	0.0		
Nalidixic Acid(30 µg)	Resistance	8	100.0	4	36.4	5	100.0	70.8	
	Sensitive	0	0.0	7	63.6	0	0.0		
Ceftriaxone(30 µg)	Resistance	3	37.5	1	9.1	1	20.0	20.8	
	Sensitive	5	62.5	10	90.9	4	80.0		
Ciprofloxacin(5 µg)	Resistance	5	62.5	2	18.	1	20.	33.3	
	Sensitive	3	37.5	9	81.8	4	80.0		
Total Isolates		8		11		5	N=24		

R%* total frequency of resistance

Discussion

In this study, drug resistance pattern of some diarrhea-causing *Enterobacteriaceae* other than

E.coli in relation to CD4 T lymphocytes counts, WHO clinical stage and Co-trimoxazole prophylaxis were assessed. Resistance pattern of some

Enterobacteriaceae isolates against co-trimoxazole was 87.5%, 90.9%, and 100% for *S. para typhi*, *Shigella* spps and *S. typhi* respectively which is in line with a study conducted in Zambia (90%) (Mwansa et al. 2002) but lower than a report from Uganda both for *Shigella* spp. This study showed over all resistance pattern of 91.7% which was higher than similar studies conducted in Northern Ethiopia (76.7%) (Asseffa et al. 1997, Huruy et al. 2011) and a study done in Uganda (83%). This periodic increment of resistance isolates with low CD4 count was also reported from Kenya (98%) (Mary et al. 2008); this might be associated with selection pressure of more resistance isolates or transfer of resistance gen between microbes (Kenneth J and Rayan C 2004)). This periodic increment of resistance isolates with

low CD4 count was also reported from a review of resistance to Nalidixic acid and Ciprofloxacin in Asia countries (64.5%) (Bing G. et al. 2012). Ampicillin were also found to be 100% resistance to *S. paratyphi* and *S. typhi* but 80.8% for isolates of *Shigella* spp which was similar to findings from Northern Ethiopia (Huruy et al. 2011). Unlike wise, Ceftraxone was sensitive for *Shigella* (90.9%) and *s. paratyphi* (62.5%) with overall sensitivity pattern of 79.2% which is lower than a study conducted in India (94%) for *S. paratyphi*. Ciprofloxacin and Chloramphenicol were found to be 81.8% and 100% sensitive for *Shigella* isolates which was lower than a finding in Rourkala India 98% and 76.6% respectively (Bhattacharya 2011) but higher than another study in Ethiopia (51.7%) for Chloramphenicol (Huruy et al. 2011). *S. typhi* were found to be the most multi drug resistance isolates with 100% resistant to Co-trimoxazole, Ampicillin, Nitrofurantoin and Nalidixic acid which was higher than a similar findings done in Ethiopia which was only 33.3% (Huruy et al. 2011) and India (12.7%). Resistance pattern for both *S. typhi* and *S. paratyphi* against Ciprofloxacin was also reported to be less than 2 %. *Shigella* spp isolates were found to be susceptible for most antimicrobial agent; 100% to Chloramphenicol and 9.1% to Co-trimoxazole which was similar to different reports else were (Threlfall and Stoberingh 2000). The overall high resistance pattern observed here could be due to overuse of this selected drug within the community. Despite extensive bacterial resistance was observed against most antibiotics, good restoration of immunity with significant improvement of CD4 T cell level were observed in individuals who were under cotrimoxazole prophylaxis and HAART for at least six months. It was in line with the report of most studies (Vajpayee et al. 1999, WHO 2006, WHO 2005).

Conclusion

In this study, extensive resistance pattern of diarrhoeagenic *Enterobacteriaceae* were observed against the antimicrobial agents of Cotrimoxazole, Ampicillin, Nalidixic acid and Nitrofurantoin. Routine treatment of acute diarrheal illness with Co-trimoxazole was questionable due to the development of high resistance pattern. Ceftriaxone and Chloramphenicol were found to be the most and relatively good drugs against common microbial isolates respectively.

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that no any computing interest. Author contribution MT involved in all designing the manuscript, writing and analysis, ZD contributed in methodological advice, editing and reviewing and BT and GD contributed in data collection and analysis of the data.

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