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MULTIDRUG RESISTANCE PROFILE AND EXTENDED-SPECTRUM BETA-LACTAMASE PRODUCTION IN FAECAL *ESCHERICHIA COLI* ISOLATED FROM HIV AND TB PATIENTS IN EKITI-STATE, NIGERIA

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ABSTRACT: *With the increasing use of antibiotics and widespread of antibiotics resistance which has been amplified by the production of ESBLs in clinical isolates; the study was to determine the multidrug resistance Profile and Extended-spectrum Beta-lactamase production in faecal Escherichia coli isolated from HIV and TB Patients in Ekiti-State, Nigeria. Three hundred isolates of E. coli were obtained from the stool samples of HIV/AIDS patients, TB patients and apparently healthy individuals. The sample was cultured on Eosin-Methylene Blue (EMB) agar plate and incubated at 37ºC for 24 hours. The colony showing greenish metallic sheen was identified using the conventional biochemical. Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion technique. Bacteria showing resistance to at least three different classes of antibiotics were considered multidrug resistant (MDR). Extended spectrum beta-lactamase production was detected by combined disc method using ceftriaxone/cefotaxime and Amoxicillin/clavulanic acid discs.A total of 141(47%) males and 159(53%) female patients were involved in the study. Based on the age distribution, age group 30-39(n=80) has the 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%), 26(52%), 21(42%) and 37(82%) resistant to SXT, AMC, SAM and AZM respectively while 24(48%), 36(72%), 23(46%) and 26(52%) of E. coli isolated from HIV patients on treatment were resistant to SXT, AMC, SAM and AZM. Tuberculosis patients on anti- TB treatment had 45(90%), 34(68%), 36(72%) and 44(88%) of the isolates resistant to SXT, AMC, SAM and AZM respectively while E. coli isolated from newly diagnosed HIV patients were 31(62%), 29(58%), 15(30%) and 27(54%) of the E. coli were resistant to SXT, AMC, SAM and AZM. Similarly, 33(66%), 36(72%), 15(30%) and 39(78%) of the isolated E. coli from newly diagnosed TB patients showed resistance to SXT, AMC, SAM and AZM respectively. Among the 148 multiple antibiotic resistant E. coli isolates 38 (23.75%) were found to be Extended β-lactamase (EBLS) positive with majority of the positive EBSL E. coli isolate from TB and TB associated patients. The increase in the prevalence of ESBL among faecal E. coli, an indicator organism for enteric pathogens, however, express the urgent need for serious antibiotics stewardship and control among clinicians and other health personnel especially in developing and under developed countries for proper management of the immune-impaired individuals.*

KEYWORD: *E. coli*, HIV/AIDS, TB, extended-spectrum beta-lactamase (ESBL)

INTRODUCTION

Antibiotic resistance is a major global public health concern (49; 40), particularly with the emergence and dissemination of antimicrobial resistance in bacteria which is well documented (39; 11). Diseases

caused by *E. coli* often require antimicrobial therapy; however, antibiotic resistant strains of *E. coli* have shown more harmful tendencies than their antibiotic susceptible counterparts. Several studies have shown sharp increase in the antibiotic resistance in *E. coli* more especially in *E. coli* O157:H7 over time (22; 29)

Bacteria have an amazing genetic flexibility that allows them to respond to a wide range of environmental threats, including the presence of antibiotic molecules that may put their existence in serious jeopardy. However, bacteria uses two major genetic strategies to resist antibiotic attack; Mutations in gene(s) often associated with the mechanism of action of the compound, and acquisition of foreign DNA coding for resistance determinants through horizontal gene transfer (45; 30; 5). The defence mechanisms within the category of antibiotic inactivation include the production of enzymes that degrade or modify the drug itself, and biochemical strategies which includes; hydrolysis, group transfer, and redox mechanisms (42). Several enzymes are known to destroy antibiotic activity by targeting and cleaving these bonds. These enzymes can often be excreted by the bacteria, inactivating antibiotics before they reach their target within the bacteria (47). One of the main mechanisms of resistance to antibacterial agents is the production of β-lactamase enzymes. In most cases, β-lactamase causes bacteria to get resistant to broad spectrum antibiotics like fluoroquinolones, aminoglycosides, trimethoprim and cephalosporin (38; 19; 30).

There is no general definition of ESBLs. A frequently used definition is that, ESBLs are β-lactamases capable of causing bacterial resistance to the penicillins; first-, second- and third-generation cephalosporins; and aztreonam (except cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by β-lactamase inhibitors such as clavulanic acid (35). *Escherichia coli* and many Gram-negative and Gram-positive bacteria produce β-lactamase enzymes and more than 200 different β-lactamases have been identified (13). β -Lactamases are classified into four groups on the basis of functional characteristics, including preferred antibiotic substrate. Clinical isolates often produce β -lactamases belonging to different functional groups (13). They can be both chromosomal and plasmid-encoded β-lactamases which can be transferred from different bacteria (28). These antibiotics have a common element in their molecular structure (a four atom ring known as a β-lactam). The lactamase enzyme breaks that ring open, deactivating the molecule's antibacterial properties. β-Lactamase is an enzyme comprised of short chains of amino acids. Its most promising use is as a catalyst for the hydrolysis and aminolysis of depsipeptides. Extended-spectrum β-lactamases (ESBLs) mediate resistance to all penicillins, third generation cephalosporins (e.g. ceftazidime, cefotaxime, ceftriaxone) and aztreonam, but not cephamycins (cefoxitin and cefotetan), carbapenems and quinolones. ESBLs are very diverse; more than 180 different ESBLs have been identified. Strains producing ESBL are commonly resistant but their resistance depends not on multiple resistance plasmids but on mutations in gyrA and parC genes, such strains are found among *E. coli* (25).

Extended-spectrum-beta-lactamases (ESBLs) are plasmid-mediated beta-lactamase of predominantly Bush class A, so far described only in gram negative bacilli (16). Extended-Spectrum-Beta-Lactamases are capable of efficiently hydrolyzing penicillin, narrow spectrum cephalosporins (cefotaxime, ceftazidine) and monobactams (aztreonam). Beta-Lactamase, inhibitors (clavulanic acid, sulbactam and tazobactam) generally inhibits ESBLs-producing strain (31). Mostly ESBLs are mutant of TEM-1, TEM-2 and SHV-1, to date none has been described that is able to hydrolyze cephamycin or carbapenems (imipenem, meropenem) (16). In addition to *Escherichia coli*, resistance to the extended spectrum cephalosporin has also been noted in *Klebsiella pneumoniae* (44). Extended-Spectrum-Beta-Lactamase antibiotics such as third generation cephalosporin (3GC) form

the major component of the empiric antibacterial chemotherapy in most clinical set ups and especially in tertiary care centers (7). Extensive use of third generation cephalosporins has contributed to the evolution of ESBL producing strains in bacteria (7). Extended- Spectrum-Beta-Lactamase also occurs predominantly in other organisms including *Salmonella spp*., *Pseudomonas aeruginosa* and other *Enterobacteriaceae* (44).

MATERIALS AND METHODS

A total of 300 samples were collected from HIV/AIDS, Tuberculosis (TB) and 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 the antibiotics profile and Extended-spectrum Beta-lactamase production among 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 , Sensitivity study was done using *E. coli* strains from stool sample collected from new TB cases, TB patients on standard Anti-TB treatment, New HIV cases, HIV patients on treatment regimen and TB/HIV negative individuals.

Culturing and Identification of Colonies

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

Antibiotics Susceptibility

Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion technique. Extended spectrum beta-lactamase production was detected by combined disc method using ceftazidime and ceftazidime/clavulanic acid discs and cefotaxime and cefotaxime/clavulanic acid discs (10). Inoculated plates were incubated overnight at 37°C for 18–24 hours, after which antibiotics sensitivity of the *E. coli* were tested using the following antibiotics disk; CIP = Ciprofloxacin (5 µg), MEM = Meropenem (10 µg), AZM = Azithromycin (15 µg), CRO= Ceftriaxone (30 µg), AMC = Amocicyllin/Cluvanic acid (20/10 µg), SXT = Sulfamethoxazole (25 µg), FEP = Cefepime (30 µg), SAM = Ampicilin/Sulbactam (10/10 µg), CXM= Cefuroxime (30 µg)

Determination of Multiple Antibiotic Resistance Index (MARI)

The frequency of multiple antibiotic resistance (MAR) has been defined as joint resistance of *E. coli* isolates to more than two antibiotics (32*).* Bacteria showing resistance to at least three different classes of antibiotics were considered multidrug resistant (MDR).The multiple antibiotic resistances (MAR) index was determined for each isolate as shown in the equation below. MAR index is the number of antibiotic(s) to which the organism is resistant divided by the total number of antibiotics tested (2).

$$\text{MARI} = \frac{\text{Number of Antibiotic (s) to which isolate was resistant}}{\text{Total number of antibiotics tested}}$$

Detection of Extended Spectrum Beta Lactamases (ESBL)

The isolates with multiple resistance to cephalosporins were screened for possible ESBL production using ceftazidime (30 µg) and cefotaxime (30 µg). According to the CLSI guidelines, the isolates showing reduced susceptibility to at least one of these drugs with zone of inhibition for ceftazidime ≤ 22 mm and cefotaxime ≤ 27 mm were considered as the possible ESBL producing strains. The

suspected ESBL producing strains were confirmed for ESBL production by combined disc assay using ceftazidime (30 µg) and ceftazidime/clavulanic acid (30/10 µg) discs and cefotaxime (30 µg) and cefotaxime/clavulanic acid discs (30/10 µg). The zones of inhibition for the ceftazidime and cefotaxime discs were compared to those of the ceftazidime/clavulanic acid and cefotaxime/clavulanic acid discs. An increase in zone diameter of ≥ 5 mm in the presence of clavulanic acid was confirmed as positive for ESBL production (10). Bacteria showing resistance to at least three different classes of antibiotics were considered multidrug resistant (27).

Statistical Analysis.
Data obtain from the research was analysed 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 30-39(n=80) followed by the ≥ 30 group.

Table 1: Age distribution of the sources of the *E. coli* isolates

Age Groups	Category of Subject					
	CI (%) n=50	HD (%) n=50	TD (%) n=50	H (%) n=50	T (%) n=50	NI (%) n=50
<30	7(14.0)	13(26.0)	12(24.0)	3(6.0)	11(22.0)	30(60.0)
30-39	14(28.0)	8(16.0)	10(20.0)	19(38.0)	15(30.0)	14(28.0)
40-49	14(28.0)	13(26.0)	10(20.0)	16(32.0)	12(24.0)	4(8.0)
50-59	11(22.0)	9(18.0)	9(18.0)	7(14.0)	6(12.0)	1(2.0)
60 and above	4(8.0)	7(14.0)	9(18.0)	5(10.0)	6(12.0)	1(2.0)

KEY: Co-infected patients =CI; HIV/AIDS patients on treatment = HD; Tuberculosis (TB) patients on treatment = TD; HIV/AIDS patients not yet on treatment regimen = H; Tuberculosis (TB) patients not yet on treatment = T; Normal Individuals (Control) = NI

Table 2: Antibiotics sensitivity of the *E. coli* isolates

Antibiotics	Category of Subject					
	CI (%) n=50	**HD (%) n=50**	**TD (%) n=50**	**H (%) n=50**	**T (%) n=50**	**NI (%) n=50**
SAM	21(42.0)	23(46.0)	36(72)	15(30.0)	15(30.0)	3(6.0)
CIP	7(14.0)	1(2.0)	5(10.0)	5(10.0)	2(4.0)	1(2.0)
AZM	35(70.0)	26(52.0)	44(88.0)	27(54.0)	39(78.0)	14(28.0)
AMC	26(52.0)	36(72.0)	34(68.0)	29(58.0)	36(72.0)	10(20.0)
FEP	0(0.0)	3(6.0)	2(4.0)	4(8.0)	2(4.0)	0(0.0)
MEM	0(0.0)	0(0.0)	0(0)	0(0.0)	0(0.0)	0(0.0)
CRO	10(20.0)	6(12.0)	12(24)	8(16.0)	16(32)	0(0.0)
CXM	0(0.0)	1(2.0.0)	0(0.0)	5(10.0)	4(8.0)	0(0.0)
SXT	40(80.0)	24(48.0)	45(90.0)	31(62.0)	33(66.0)	15(30.0)

KEY: Co-infected patients =CI; HIV/AIDS patients on treatment = HD; Tuberculosis (TB) patients on treatment = TD; HIV/AIDS patients not yet on treatment regimen = H; Tuberculosis (TB) patients not yet on treatment = T; Normal Individuals (Control) = NI
CIP=Ciprofloxacin (5 µg); MEM = Meropenem (10 µg); AZM = Azithromycin (15 µg); CRO = Ceftriaxone (30 µg); AMC = Amoxicillin/Clavulanic acid (20/10 µg), SXT = Sulfamethoxazole (25 µg); FEP=Cefepime (30 µg); SAM = Ampicillin/Sulbactam (10/10 µg); CXM = Cefuroxime (30µg)

Multiple Antibiotic Resistance Index

The multiple antibiotic resistance (MAR) indices obtained from this study as shown in Table 2 were 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 respectively.Table 2 indicates the multiple antibiotic resistance of the isolated E. coli to different classes of antibiotics, 10% of the isolates were resistant to a single class of antibiotics > 82(27.33%) were resistant to two classes of antibiotics, 72(24%) of the isolates were resistant to three classes of antibiotics while 54(18%) of the isolates were resistant to four classes of antibiotics, 18(6%) of the isolates were resistant to five classes of antibiotics and 2(0.7%) of the isolates were resistant to 6 classes of antibiotics and at this level, there is a significant level of misuse of these antibiotics. In Nigeria, even prescription drugs can readily be obtained over the counter without prescription from a clinician. Antibiotics is said to be abused if the MAR Index is ≥0.2 in a

particular environment which can add to the increase of drug resistance worldwide (46); hence, threatening the ability to effectively treat patients. *E. coli* isolated from HIV/TB co-infected reveals 40(80%), 26(52%), 21(42%) and 37(82%) resistant to SXT, AMC, SAM and AZM respectively while 24(48%), 36(72%), 23(46%) and 26(52%) of *E. coli* isolated from HIV patients on treatment were resistant to SXT, AMC, SAM and AZM. Tuberculosis patients on anti- TB treatment had 45(90%), 34(68%), 36(72%) and 44(88%) of the isolates resistant to SXT, AMC, SAM and AZM respectively while *E. coli* isolated from newly diagnosed HIV patients were 31(62%), 29(58%), 15(30%) and 27(54%) of the *E. coli* were resistant to SXT, AMC, SAM and AZM. Similarly, 33(66%), 36(72%), 15(30%) and 39(78%) of the isolated *E. coli* from newly diagnosed TB patients show resistance SXT, AMC, SAM and AZM respectively. Ciprofloxacin, cefuroxime, ceftriaxone and Cefepime showed reasonable sensitivity to the *E.coli* isolate while Meropenem was sensitive to all the isolates.

Table 3: Frequency of multiple antibiotic resistance (MAR) and multiple antibiotic resistance indices of *E.coli* isolates from HIV/AIDS and TB patients in Ekiti State, Nigeria.

S/N	Resistance pattern	Antibiotic classes	Numbers	MAR Index
1	SAM,	1	11	0.1
2	SXT,	1	10	0.1
3	AMC	1	8	0.1
4	AZM	1	12	0.1
5	AZM, AMC	2	15	0.2
6	AZM, SXT	2	20	0.2
7	SXT, SAM	2	9	0.2
8	AMC, SXT	2	12	0.2
9	AMC, SAM	2	3	0.2
10	AZM, SAM	2	6	0.2
11	CRO, SAM	2	1	0.2
12	CRO, AMC	2	2	0.2
13	AMC, CXM	2	5	0.2
14	AMC, CRO	2	1	0.2
15	CRO, SXT	2	1	0.2
16	MEM, FEP	2	1	0.2
17	AMC,SXT,SAM	3	12	0.3
18	AZM,AMC,SAM	3	16	0.3
19	AZM,AMC,SXT	3	23	0.3
20	AMC,CXM,SXT	3	2	0.3
21	CIP,AMC,SAM	3	1	0.3
22	AMC,FEP,CRO	3	1	0.3
23	AZM,FEP,SXT	3	2	0.3
24	CRO,SXT,SAM	3	1	0.3
25	AZM,SXT,SAM	3	9	0.3
26	AZM,AMC,CXM	3	2	0.3
27	AZM,AMC,CRO	3	2	0.3
28	AZM,FEP,SAM	3	1	0.3
29	FEP,SXT,SAM	3	1	0.3
30	CIP,AZM,AMC	3	3	0.3

31	AZM,CXM,SXT	3	1	0.3
32	AZM,CRO,SXT	3	1	0.3
33	AZM,AMC,SXT,SAM	4	23	0.4
34	CIP,AZM,SXT,SAM	4	1	0.4
35	AZM,FEP,SXT,SAM	4	4	0.4
36	AMC,CRO,SXT,SAM	4	1	0.4
37	AMC,FEP,SXT,SAM	4	1	0.4
38	AZM,AMC,CRO,SAM	4	2	0.4
39	AZM,AMC,CXM,SXT	4	2	0.4
40	CIP,AZM,AMC,SXT	4	9	0.4
41	AZM,FEP,CRO,SXT	4	1	0.4
42	AZM,AMC,FEP,CXM	4	1	0.4
43	AMC,FEP,CRO,SXT	4	1	0.4
44	AZM,MEM.SXT,SAM	4	1	0.4
45	AMC,FEP,SXT,SAM	4	1	0.4
46	AZM,CRO,SXT,SAM	4	1	0.4
47	AZM,AMC,FEP,SXT	4	1	0.4
48	AZM,AMC,CRO,SXT	4	1	0.4
49	AZM,CRO,SXT,SAM	4	1	0.4
50	AZM,FEP,CXM,SAM	4	1	0.4
51	AZM,AMC,CRO,SXT,SAM	5	3	0.5
52	AZM,AMC,CXM,SXT,SAM	5	2	0.5
53	CIP,AMC,CRO,SXT,SAM	5	4	0.5
54	AZM,AMC,FEP,SXT,SAM	5	2	0.5
55	CIP,AZM,AMC,SXT,SAM	5	3	0.5
56	CIP,AZM,FEP,SXT,SAM	5	1	0.5
57	CIP,AZM,AMC,CXM,SXT,SAM	6	1	0.6
58	AZM,AMC,FEP,CXM,SXT,SAM	6	1	0.6

Key: CIP=Ciprofloxacin (5 µg); MEM = Meropenem (10 µg); AZM = Azithromycin (15 µg); CRO = Ceftriaxone (30 µg); AMC = Amoxicillin/Clavulanic acid (20/10 µg), SXT = Sulfamethoxazole (25 µg); FEP=Cefepime (30 µg); SAM = Ampicillin/Sulbactam (10/10 µg); CXM = Cefuroxime (30µg)

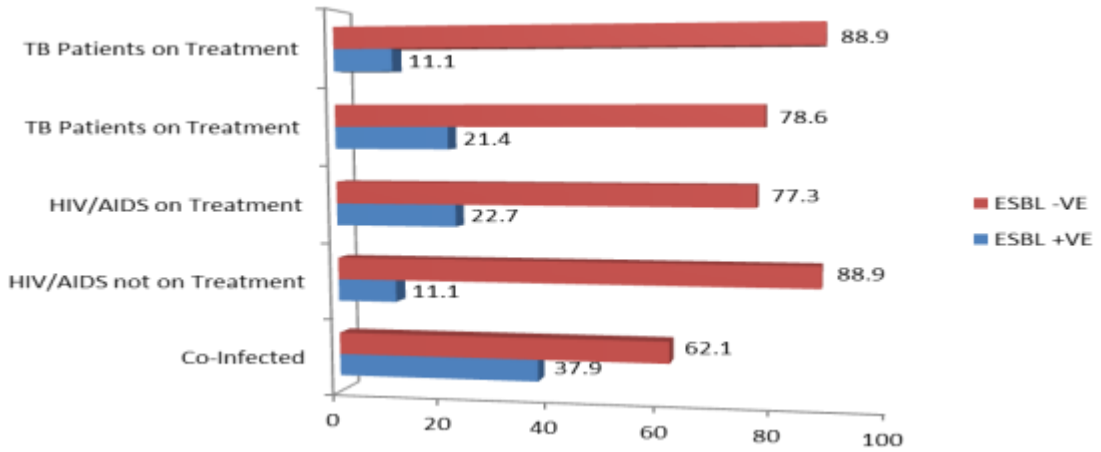

Figure 1. Prevalence of Extended Spectrum Beta Lactamases (ESBL)

The above figure shows the frequency of positive ESBL producing isolates from all the groups. This revealed that majority (29 of 38) of the ESBL producing isolates are from TB and TB associated groups with five ESBL producing isolates were from HIV/AIDS patient that are on treatment, and three ESBL producing isolates were isolated from newly diagnosed HIV patients while none of the isolates from normal individuals is positive to ESBL production Among the 148 multiple antibiotic resistant *E. coli* isolates 38 (23.75%) were found to be Extended β-lactamase (EBLS) positive with majority of the positive EBSL *E. coli* isolate from TB and TB associated patients while 122 (76.25%) were found to be Extended β-lactamase (EBLS) negative. This high prevalence of ESBL positive isolates is in line with the studies conducted in Kano on the prevalence of Extended Spectrum Beta Lactamases (ESBL) Producing *Escherichia coli* and *Klebsiella pneumoniae* in Tuberculosis patients in Kano and another study done in Lagos, both in Nigeria. They got the overall prevalence of ESBL among tuberculosis patient to as 37.1% and 20% respectively (1, 52).

The increase in the prevalence of ESBL among faecal *E. coli*, an indicator organism for enteric pathogens, however, express the urgent need for serious antibiotics stewardship and control among clinicians and other health personnel especially in developing and under developed countries for proper management of the immune-impaired individuals. As a result, ESBL-producing organisms pose a major problem for clinical case management. The multidrug resistance profiling of the *E. coli* isolates showed a high prevalence of resistance to most of the commonly used antibiotics. A possible pressure imposed by the use of these antibiotics in human medicine for prophylaxis or case management, is a major factor in antimicrobial resistance in *E. coli*. This however calls for a need to enact and enforce laws to limit the prescription and dispensing of antibiotics to only qualified professionals. Also vigorous and continuous education of the public on the dangers of reckless use and purchase of antibiotics is also very important.

CONCLUSION

This work reveals that faecal *E. coli*, an indicator organism for enteric pathogens, rapidly develops resistance to different classes of antibiotics with an increase in the prevalence of ESBL among faecal *E. coli* increase in the production of ESBL most especially among tuberculosis patients studied. This raises the fear of rapid spread of antibiotic resistance strains in hospital and communities. The reliance on common antibiotics especially the third generation cephalosporins in treating infections caused by ESBL producers may result in treatment failure and thus leading to economic stress and further complicate the health condition of immuno-compromised HIV and TB patients.

It is therefore recommended that adequate attention and routine screening for ESBL should be carry out in all hospital laboratories to prevent its rapid spread among patients in the in the hospital community. Similarly, urgent need for serious antibiotics stewardship and control among clinicians and other health personnel especially in developing and under developed countries for proper management of the immune-impaired individuals.

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