ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Occurrence of extended spectrum beta-lactamase producing gram negative bacteria in HIV AIDS infected patients with urinary and gastrointestinal tract infections in Benin metropolis.

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ABSTRACT

This study was carried out to determine the prevalence of extended spectrum beta-lactamase (ESBL) producing gram negative bacteria associated with HIV/AIDS infected individuals in Benin Metropolis, Edo state, Nigeria. A total of 948 samples (603 urine and 345 diarrhoea stools) were collected from individuals living with HIV/AIDS between October 2009 and October 2010. A total of 260 gram negative bacteria were isolated from the samples. Sensitivity studies were carried out using disc diffusion method by Kirby-Bauer and phenotypic characterisation of ESBL was carried out using double disc synergy test (DDST). The result of the study revealed that 45 (17.3%) were positive for ESBL production, 13 (28.9%) were from *Escherichia coli,* 9 (20.0%) from *Klebsiella pneumoniae,* 7 (15.6%) from *pseudomonas aeruginosa,* 5 (11.1%) from *serratia marcescens,* 3(6.7%) from *Salmonella* spp, 5(11.1%) from *Proteus* spp, 2(4.4%) from *Citrobacter* spp and 1(2.2%) from *Enterobacter* spp. Resistance patterns of ESBL producers to cephalosporins revealed that they are multi-drug resistant. This study therefore, do not only proclaim the presence of ESBL producing gram negative bacteria in HIV/AIDS patients, but also emphasises that they are highly resistant to the cephalosporins as such it is important to carry out screening for ESBL producers among gram negative bacteria isolates from HIV/AIDS patients.

Keywords: ESBL, HIV/AIDS, cephalosporins, antibiotics. gram negative Bacteria , Benin Metropolis.

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ISSN: 0975-8585

INTRODUCTION

The introduction of antimicrobial drugs most notably the cephalosporins was thought to herald the beginning of the end of bacterial infections. Resistance of gram negative bacteria to the cephalosporins such as oxyimino-beta-lactams was first described in 1980 and since then a linear increase in resistance has been recorded. Their resistance is by the production of extended spectrum beta lactamase (ESBL) [1]. ESBL are encoded by transferable conjugative plasmids which often encode resistant determinants to other classes of antibiotics. ESBLs are mostly the products of point mutations at the active site of TEM and SHV enzymes [2]. Majority of ESBL – producing organisms are *E. coli* and *k. pneumoniae* others includes *Enterobacter* spp, *Salmonella* spp, *Serratia marcescens*, *Morganella* spp, *Proteus mirabilis* and *Pseudomonas* spp [2].

Infections with opportunistic pathogens have been one of the hallmarks of the acquired immunodeficiency syndrome since the beginning of the epidemic. Little attention has been given to the role of ESBL – producing gram negative bacteria infections in complications associated with HIV infections. Urinary and gastrointestinal infections account for a sizeable number of these opportunistic infections in hospitalised HIV – infected individuals in developing countries [3, 4].

The increased predisposition of HIV/AIDS patients to invasive bacterial isolates has been described but there are no detailed studies or literatures on the prevalence of ESBL producing gram negative bacteria in HIV/AIDS infected individuals in Benin Metropolis. This study is therefore designed to look at the occurrence of ESBL producing Gram negative bacteria in HIV infected individuals in Benin Metropolis.

MATERIALS AND METHODS

A total of 260 gram negative bacteria were isolated from603 urine and 345 diarrheal stool samples of HIV/AIDS patients attending University of Benin Teaching Hospital (UBTH), Benin City, Edo state, Nigeria from October 2009 to October 2010. The urine specimens were inoculated aerobically into MacConkey and Cled agar plates and the diarrheal stools inoculated into Blood agar, MacConkey, Deoxycholate Citrate agar and Selenite F at 37^0C for 24 to 48h. The colonies of each representative isolates where then characterised using standard bacteriological methods [5]. They were further sub-cultured and stored on nutrient agar slants at 4^0C for further analysis.

The susceptibility of the bacteria isolates to cefotaxime (30µg), ceftazidime (30µg), and Ceftriaxone (30µg) was studied by Kirby-Bauer disc diffusion method according to NCCLS on Muller-Hinton agar (Difco Laboratories, Detroit, Mich USA) (NCCLS, 12000).

ESBL was detected using the Double disc Synergy test (DDST). Synergy was determined between a disc Augmentin (20µg amoxicillin+10µg clavulanic acid) and 30µg of disc of

ISSN: 0975-8585

cefotaxime and ceftazidime antibiotics placed a distance of 15mm apart from the Centre disc on the surface of culture of the resistant isolate under test on Mueller-Hinton agar. Inoculated media were incubated for 24 hours at 37^0C. The test organisms were considered to produce ESBL if the zone size around the test antibiotic disc were more than 5mm and above towards the augmentin disc.

RESULTS

A total of 603 urine and 345 diarrhoea stool samples were collected from HIV/AIDS patients to determine the production of ESBL by gram negative bacteria isolates. A total of 260 gram negative bacteria, *E. coli* 66(25.4%), *Klebsiella pneumoniae* 60(23.0%), *Pseudomonas aeruginosa* 50 (19.2%), *Serratia marcescens* 45(17.3%) *Salmonella* spp 19(7.3%), *Proteus* spp 35(13.5%), *Citrobacter* spp 23(8.9%) and Enterobacter 7(2.7%) were isolated; 109(41.9%) were from urine samples while 151(58.1%) were from diarrhoea stools samples of HIV/AIDS infected patients (Table I).

The result of the present study showed that out of 260 gram negative bacteria isolated 45(17.3%) showed evidence of ESBL production (Table I). *Escherichia coli* yielded the highest percentage of ESBL producers. Table 2 shows the result of the susceptibility of the gram negative bacteria isolates to ceftazidime, cefotaxime and ceftriaxone. It was observed that ESBL organisms from all the specimens were generally resistant to the cephalosporins.

Table I: Prevalence of ESBL producing gram negative bacteria from urine and diarrhea stool samples of HIV/AIDS patients in Benin Metropolis.

	Total number of Isolates (%)	Total ESBL positive isolates (%)
Escherichia coli	66(25.4)	13(28.9)
Klebsiella pneumoniae	60(23.0)	9(20.0)
Pseudomonas aeruginosa	50(19.2)	7(15.6)
Serratia marcescens	45(17.3)	5(11.1)
Salmonella spp	19(7.3)	3(6.7)
***Proteus* spp**	35(13.5)	5(11.1)
***Citrobacter* spp**	23(8.9)	2(4.4)
***Enterobacter* spp**	7(2.7)	1(2.2)
Total	206	45

Table II: Percentage (%) resistance of gram negative bacteria isolated from HIV/AIDS patients in Benin metropolis to the cephalosporins.

	No of Isolates		CXM	CAZ	CRO
Escherichia coli	66		50(75.8)	47(71.2)	49(74.2)
Klebsiella pneumoniae	60		43(71.7)	40(66.7)	41(68.3)
***Pseudomonas* spp**	50		31(62.0)	21(42.0)	27(54.0)
Serratia marcescens	45		29(64.4)	23(51.1)	26(57.8)
***Salmonella* spp**	19		9(47.4)	5(26.3)	6(31.6)
***Proteus* spp**	35		20(57.1)	12(34.4)	18(51.4)
***Citrobacter* spp**	23		11(47.8)	4(17.3)	7(30.4)
***Enterobacter* spp**	7		2(28.6)	1(14.3)	2(28.6)

Key: CXM-Cefotaxime, CAZ-Ceftazidime, CRO-Ceftriaxone

ISSN: 0975-8585

DISCUSSION

Several studies have shown that there is a synergy between opportunistic pathogens and HIV [6, 7]. It has also been shown that these pathogens breakdown the mucosal barrier, thereby permitting easier accesses of the HIV to blood and consequently to the T helper's and immune cells [8]. The present study has shown such detrimental association between HIV/AIDS and gram negative bacteria of the urinary and gastrointestinal tract. In this study, 45(17.3%) of the gram negative bacteria from urine and diarrheal stool were shown to produce ESBL. These isolates were found to be resistant to the cephalosporins at varying degrees.

The result of this investigation shows that organisms harbouring ESBL enzymes are multi-drug resistant and thus, could pose serious treatment challenges. The presence of ESBL on plasmid makes it possible for them to be easily transferred from one organism to another. The overall result of the present study shows that while the prevalence of ESBL producing gram negative bacteria isolates from HIV/AIDS patients in Benin Metropolis is currently not very high, it may escalate and cause a very serious public health problem especially in patients that are antiretroviral naive, if not checked in good time. The constant use of third generation cephalosporins in the treatment of infections is probably the reason for the current spread of ESBL producing organisms in the environment. Also, it is a known fact that HIV complicates the treatment of infections by increasing mortality and morbidity during antibiotic treatment and also increasing the risk of recurrent infections after the treatment has been successfully completed [6].

In conclusion, the results of our work shows that 45(17.3%) of the gram negative bacteria obtained from urine and diarrhoea stool of HIV infected patients in Benin metropolis produced ESBL. *E. coli* 13(28.9%) was the most prevalent ESBL producer and *Enterobacter* spp 1(2.2%) was the least producer of ESBL. ESBL producing gram negative bacteria isolated from urine and stool specimens showed differences in their rate of susceptibility to the cephalosporins.

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