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PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASES (ESBL) PRODUCING *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* IN TUBERCULOSIS PATIENTS IN KANO, NIGERIA

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ABSTRACT

Resistance to broad spectrum β lactams, mediated by extended spectrum beta lactamase (ESβL) is an increasing problem worldwide. Production of these enzymes in clinical infections can result in treatment failure if one of the second or third generation cephalosporins is used. This study investigates the incidence of ESBL among E. coli and K. pneumoniae which were isolated from tuberculosis patients with secondary opportunistic bacterial infection attending Aminu Kano Teaching Hospital (AKTH), Kano and Infectious Disease Hospital (IDH), Kano. A total of 37 E. coli and 33 K. pneumoniae obtained from their sputum were screened for ESBL production by Double disk synergy test method (DDST). Prevalence of 37.3% (14/37) and 36.4% (12/33) was recorded for Escherichia coli and Klebsiella pneumoniae respectively. Furthermore, a slight high prevalence of 39.4% (13/33) was recorded with the female tuberculosis patients when compared with their male counterpart 35.1% (13/37). Escherichia coli harboring ESBL were more encountered among the elderly patients aged 31-50 (13/51 or 25.5%) when compared with K. pneumoniae with (9/51 or 17.6%). The study shows alarming rise in ESBL production among Klebsiella pneumoniae and Escherichia coli among immunocompromised patients raising fear of possible emergence of multiple drug resistant bacteria that will be hard to treat. Thereby early detection of ESBL in these patients is recommended to curb the spread,

Keywords: Extended spectrum beta lactamase (ESBLs), Escherichia coli, Klebsiella pneumoniae, Tuberculosis.

INTRODUCTION

The production of Beta-lactamase is an important mechanism of resistance to Beta-lactam antibiotics among gram negative bacteria (Coudron, 1997). Extended-spectrum-beta-lactamases (ESBLs) are plasmid-mediated beta-lactamase of predominantly Bush class A, so far described only in gram negative bacilli (Emery and Weymouth, 1997). 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 inhibit ESBLs-producing strain (Naumoski and Paizkill, 1996).

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) (Emery and Weymouth, 1997). In addition, resistance to the extended spectrum cephalosporin has also arisen in *Klebsiella pneumoniae* and *Escherichia coli* (Spanu *et al.,* 2002). 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 (Chaudary and Aggarwal, 2004). Extensive use of third generation cephalosporins has contributed to the evolution of ESBL producing strains in bacteria (Chaudary and Aggarwal, 2004). Extended-Spectrum-Beta-Lactamase also occurs predominantly in other organisms including *Salmonella spp.*, *Pseudomonas aeruginosa* and other *Enterobacteriaceae* (Spanu *et al.,* 2002).

The ESBLs are frequently plasmid encoded (Phillippon *et al.,* 2002). Plasmid responsible for ESBL production frequently carries gene encoding resistance to other drug classes (for example, amino glycoside) (Phillipon *et al.,* 2002). Therefore, antibiotic options in the treatment of ESBL producing organisms are extremely limited (Emery and Weymouth, 1997). ESBL producing organism may appear susceptible to some extended-spectrum cephalosporins *in vitro* (Naumorski, and Paizkill 1996). However, treatment with such antibiotics has been associated with high failure rate (Bradford, 2001).

Gram negative *Enterobacteriaceae* expressing ESBLs are among the most multidrug-resistant pathogens in hospitals and are spreading worldwide. Infections caused by ESBL-producing organisms have resulted in poor prognosis, prolonged hospital stay and greater hospital expenses (Lautenbach *et al.,* 2001 and Paterson *et al.,* 2004). Physical contact is the most likely mode of transmission.

The gastrointestinal tract of colonized or infected patients is the most frequent reservoir (Paterson *et al.,* 2004). Several studies (Paterson *et al.,* 2004) indicate that transient carriage of bacterial on the hands of healthcare workers (HCWs) may lead to transmission to patients.

Tuberculosis (TB) is a common and often deadly infectious disease caused by various strains of Mycobacteria, usually *Mycobacterium tuberculosis* in human (Jasmer and Nahid, 2002). Tuberculosis usually attacks the lung but can also affect other parts of the body. It is spread through the air when people who have the disease cough, sneeze or spit (Southwick, 2007).

The multi resistant phenotype emerges with sequential acquisition of mutations in several loci of separate gene (WHO, 2010 and Southwick*,* 2007). Knowledge of this mechanism of resistance permits the development of techniques for the early detection of resistance strains, thereby making proper control possible (Southwick*,* 2007).

It has been found that tuberculosis patients whose immune system is compromised prone to many secondary bacterial infections. Some of the bacterial capable of causing such infections were found to be resistance to many antibiotics such as cefazolin, ceftazidine, imipenem, carbenicillin by producing enzymes such as extended-spectrum-beta-lactamases (ESBLs). Such isolates may cause serious opportunistic infections especially in tuberculosis patients. Resistance to such enzymes in clinical isolate has lead to little or no treatment option which consequently leads to death. Therefore this study aimed at determining the occurrence of ESBL producers among *E. coli* and *K. pneumoniae* which often cause secondary bacterial infections in Tuberculosis patients and detect if transfer of resistant genes are possible between these bacteria of different genera.

MATERIALS AND METHOD

Clinical Isolates

A total of seventy isolates including 37 *E. coli* and 33 *K. pneumoniae* were isolated from 138 sputum samples of tuberculosis infected patients attending Tuberculosis laboratory of Aminu Kano Teaching Hospital (AKTH), Kano and Infectious Diseases Hospital (IDH), Kano over a period of three (3) months (September-November, 2010). The patients are confirmed to be tuberculosis patient by detecting acid fast bacilli (AFB) using Ziehl Nelson (ZN) stain (Cheesbrough, 2002). Secondary bacterial pathogens were isolated from their sputum using standardized microbiological techniques. Identification of the *E. coli* and *K. pneumoniae* pathogens was done using microscopic and biochemical examinations (Cheesbrough, 2002).

Detection of Extended Spectrum Beta Lactamases (ESBL)

The double disc synergy test (DDST) method described by NCCLS (2000) was employed. Standardized inoculums of the test organisms were inoculated on Muellar Hinton Agar (MHA) (Oxoid, England) using sterile swab stick. Amoxicillin/clavulanic acid disc (30ug, Oxoid, England) was placed at the center of the inoculated MHA. Ceftazidime (30ug, Oxoid , England) and Cefpodoxime (10ug, Oxoid , England) were placed 15mm center to center from the Amoxycillin/clavulanic acid discs. The plates were incubated at 37^{o}C for 24 hours. After incubation, enhancement of zone of inhibition of either or both the Ceftazidime and Cefpodoxime discs towards the Amoxycillin/Clavulanic acid discs is indicative of ESBL production. The enhancement is due to inhibition of ESBL by clavulanic acid and subsequent action of the extended spectrum cephalosporins.

Transconjugation Test

The method adopted by Iroha *et al*., (2010) was used. One ESBL producing isolates each of E. coli and *K. pneumoniae* were selected for bacterial conjugation. The ESBL producing *E. coli* (donor) was transconjugated with 3 none ESBL producing *K. Pneumoniae* (recepient), while ESBL producing *K. pneumoniae* (donor) was transconjugated with 3 none ESBL *E. coli* (recepient). The recipient and the donor strains were grown separately in 5ml of a double strength Nutrient broth (Oxoid, England), incubated at 37^{0}C for 24 hours. The cultures of donor and recipient were mixed at a ratio of 1:10 (donor: recepient) and incubated at 37^{0}C for 3 hours. Samples of the mixture were inoculated on a differential media MacConkey (Hi-media, India) and incubated at 37^{0}C for 24 hours. The colonial appearances of the mixed colonies of both *E. coli* and *K. pneumoniae* were carefully studied and the isolates were subjected to biochemical tests in accordance with the method described by Cheesbrough, 2002 for identification. The transconjugants were later screened for ESBL.

RESULTS AND DISCUSSION

The overall prevalence of ESBL among tuberculosis patient is 37.1% (Table 1). *Escherichia coli* recorded a prevalence of 37.8% followed by *Klebsiella pneumoniae* with 36.4% prevalence (Table 1). This prevalence is alarming when compared with the findings of Aibinu *et al*. (2003) who reported 20% prevalence in Lagos-Nigeria, Kader and Kumar, (2005) who found 4.8% in Saudi Arabia and Gangoue-Pieboji, (2005) who reported a prevalence rate of 12% in Younde, Cameroun, El-Khizzi and Bakheshwain, (2006), 15.8% in Riyadh, Saudi Arabia and Yushau *et al*. (2007) 9.3% prevalence in Kano Nigeria. Though their reports did not specify if they were immunocompromised or not, but Jayapradha *et al*., 2007 reported a prevalence of 12% among tuberculosis patients in India.

The nearly similar prevalence of 36.4% and 37.8% for *K. pneumoniae* and *E. coli* in this study also agreed with the findinds of Babypadmini and Appalaraju (2004), who reported 40 and 41% ESBL positivity among *K. pneumoniae* and *E. coli*, respectively, but a more higher prevalence of 86% for *Klebsiella pneumoniae* and 40% for *Escherichia coli* in tuberculosis patients was reported by Jayapradha *et al*., in 2007.

The present study revealed a slightly higher recovery of ESBL producers in males (*E. coli*, 38.9%; *K. pneumoniae*, 40%) than in females (*E. coli*, 36.8%; *K. pneumoniae* 33.3%). This was incomparable with other findings which reported a higher preponderance in females. The age wise distribution of the ESBL producers among the patients showed the highest prevalence among the 51-above years age group (33.3%). This is in accordance with findings of Kiratisin *et al*., (2008). This may be because of the increased hospitalization of the patients with ages around 60 years in the medical and surgical units due to their weaker immunity.

The knowledge of the resistance pattern of the bacterial strains in tuberculosis patients will help in guiding an appropriate and judicious antibiotic use putting into consideration their already compromised immune status. There is a possibility that a restricted use of antibiotics can lead to the withdrawal of the selective pressure and that the resistant bacteria will no longer have a survival advantage in such settings.

This study also revealed transfer of resistant genes between ESBL producing *E. coli* and *K. pneumoniae* to non ESBL producing *K. pneumoniae* and *E. coli* respectively (Table 4). Shi *et al*., (2009) also reported similar transfer between *K. pneumoniae* and *E. coli.* High rate of this transfer occurred between *E. coli* and *K. pneumoniae* (66.7%) than vice verca (33.3%). This indicated that plasmid carrying ESBL gene in one bacteria can spread rapidly to members of the same species or organisms of different genera in the same or different individual. Furthermore, the study also revealed the possibility of transfer of antibiotic resistance genes between pathogens and normal flora of the upper respiratory tract which under immune-suppressed condition can cause infections that may be difficult to treat.

Table 1: Prevalence of ESBL among *Escherichia coli* and *Klebsiella pneumoniae in* Tuberculosis patients.

S/N	Clinical isolates	No of isolate screened	No. of positive ESBL	% Prevalence
1.	*Escherichia coli*	37	14	37.8
2.	*Klebsiella pneumoniae*	33	12	36.4
	Total	70	26	37.1

Table 2: **Sex distribution of patients in whom ESBL isolates were detected.**

S/N	Clinical isolates	Male		Female	
		No screened (%)	No. of positive ESBL (%)	No screened (%)	No. of positive ESBL(%)
1	*E. coli*	18(54.5)	7(38.9)	19(51.4)	7(36.8)
2	*K. pneumoniae*	15(45.6)	6(40.0)	18(48.6)	6(33.3)
	Total	33(100)	13(78.9)	37(100)	13(70.1)

Table 3: **Age distribution of patients in whom ESBL were detected**

Age group (years)	No of occurrence	No positive for ESBL and %			
		E. coli	%	*K. pneumoniae*	%
1-10	0	0	0	0	0
11-20	6	0	0	1	8.3
21-30	13	1	7.1	2	16.7
31-40	12	4	28.6	3	25.0
41-50	14	4	28.6	2	16.7
51-Above	25	5	35.7	4	33.3
Total	70	14	53.8	12	46.2

Table 4: Transconjuction of resistant gene between ESBL and non ESBL producing strains

S/N	Donor strain (N=1)	Recipient strain (N=3)	No of successful transconjugation	%
1.	*E. coli*	*K. pneumoniae*	2	66.7
2.	*K. pneumoniae*	*E. coli*	1	33.3

CONCLUSION

In conclusion, ESBL occurred among secondary bacterial pathogens of *E. coli* and *K. pneumoniae* in tuberculosis patients studied. Similarly, transfer of plasmid carrying resistant genes occurred between the two pathogens isolated from the patients. This raises the fear of rapid transmission of antibiotic resistance strains in hospital and communities acquired nosocomial infections. The reliance on empirical chemotherapy (especially with third generation cephalosporins) of infections caused by ESBL-producers may result in treatment failure and thus complicate the health condition of immuno-compromised (TB) patients.

It is therefore recommended that adequate screening for ESBL should be carry out in all hospitals to prevent its rapid spread among patients in the same ward or under same care by a medical officer or team. Hand washing technique between each patients by the medical personel should be enhanced to prevent patient-patient transfer by the medical personnel.

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