academicJournals

Vol. 8(43), pp. 3687-3694, 22 October, 2014
DOI: 10.5897/AJMR2014.6931
Article Number: 75538F948276
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Extended-spectrum β-lactamase production and antimicrobial resistance in *Klebsiella pneumoniae* and *Escherichia coli* among inpatients and outpatients of Jimma University Specialized Hospital, South-West, Ethiopia

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Received 30 May, 2014; Accepted 6 October, 2014

Extended spectrum β-lactamases (ESBLs) have emerged as a major threat worldwide with limited treatment options. The prevalence of ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* strains largely remain unknown in Ethiopia. The study was aimed at determining the occurrence of extended spectrum β-lactamase-producing *E. coli* and *K. pneumoniae* among inpatient and outpatient settings, their antimicrobial resistance profile and associated risk factors in Jimma University Specialized Hospital (JUSH). A total of 471 consecutive, non repetitive clinical specimens were collected among inpatients (n=314) and outpatients (n=157). Among these, 112 isolates of *K. pneumoniae* (n=27) and *E. coli* (n=85) were recovered. Overall prevalence of extended spectrum beta lactamase (ESBL) producers was 38.4% (n=43) of total isolates. Extended spectrum beta lactamases were found in 28.2% (n=24) of *E. coli* and 70.4% (n=19) of *K. pneumoniae*. Extended spectrum beta lactamase producers mediated very high resistance to both beta-lactams and non-betalactams, and they were significantly higher among in-patients (46.4%) than out-patients (14.3%). On Multivariate analysis, treatment with third generation cephalosporin was identified as a sole risk factor for acquisition of ESBL enzyme. Our findings confirmed that infection due to extended spectrum beta lactamase-producing *E. coli* and *K. pneumonia* is prevalent in JUSH and that exposure to third generation cephalosporin was associated with these infections. The magnitude of *E. coli* and *K. pneumoniae* infection was more in inpatients with higher levels of extended spectrum beta lactamase production than outpatients.

Key words: *Escherichia coli*, *Klebsiella pneumoniae,* extended spectrum β-lactamases, inpatients, outpatients.

INTRODUCTION

The problem of microbial drug resistance has achieved a global dimension and an alarming magnitude, being one of the leading unresolved problems in public health. The relentless evolution of resistance, in the face of a decrease in the development of new antimicrobial agents active against resistant pathogens, has led to an increa-

sing number of cases in which the pathogen is resistant to most, or even all, drugs available for clinical use (Rossolini and Mantengoli, 2008). β-Lactam agents such as penicillins, cephalosporins, monobactams and carbapenems are among the most frequently prescribed antibiotics worldwide (Pitout et al., 2005). β-Lactams account for approximately 50% of global antibiotic consumption (Livermore, 1998). Bacterial resistance to β-lactam antibiotics occurs by three mechanisms: failure of the β-lactam to reach the penicillin-binding proteins (PBPs), low-affinity binding to the PBPs and inactivation of the drug by β-lactamases (Holbrook and Lowy, 1998). Among this, β-lactamases are the commonest cause of bacterial resistance to β-lactam antimicrobial agents (Livermore, 1995).

The introduction of the third-generation cephalosporins into clinical practice in the early 1980s was heralded as a major breakthrough in the fight against β-lactamase-mediated bacterial resistance to antibiotics. The first report of plasmid-encoded β-lactamases capable of hydrolyzing the extended-spectrum cephalosporins was published in 1983 in Germany (Lautenbach et al., 2001). Later these enzymes were named extended-spectrum β-lactamases (ESBLs) (Shah et al., 2004). Since then, several outbreaks have been reported in a number of European countries and the USA, and the problem has reached endemic dimensions in several places worldwide (Giamarellou, 2005).

An extended-spectrum β-lactamase is any β-lactamase that can confer resistance to the oxyiminocephalosporins (e.g. cefotaxime, ceftriaxone, ceftazidime) and monobactams (e.g. aztreonam), but not to the cephamycins (e.g. cefoxitin and cefotetan) and carbapenems (e.g. imipenem, meropenem, and ertapenem), and which can be inhibited by β-lactamase inhibitors such as clavulanic acid (Pitout and Laupland, 2008). ESBLs are known as extended-spectrum because they are able to hydrolyze a broader spectrum of β-lactam antibiotics than the simple parent β-lactamases from which they are derived (Al-Jasser, 2006). More than 500 variants of ESBL have been described and the majority of these belong to the Temoniera (TEM), sulfhydryl variable (SHV) and Cefotaximase-Munich (CTX-M) family (http://www.lahey.org/studies/webt.htm).

K. pneumoniae and *E. coli* remain the major ESBL-producing organisms isolated worldwide, but these enzymes have also been identified in several other members of the Enterobacteriaceae family (Pitout and Laupland, 2008). ESBL-producing *E. coli* and and *K. pneumoniae* (ESBL-EK) pathogens are of great concern for many reasons. First, ESBL-EK isolates are often difficult to treat because they carry plasmids that confer resistance to multiple antibiotics. Second, patients with ESBL-EK infections may experience a delay in appropriate therapy because current methods of identification can leave them undetected. Third, patients with ESBL-EK infections have significantly longer hospital stays and incur greater hospital charges than do patients without these infections. Finally, patients with ESBL-EK infections have an increased risk of death when compared with patients with non-ESBL-EK infections (Bisson et al., 2002). A recent report from the Infectious Diseases Society of America listed ESBL-producing *Klebsiella* spp. and *E. coli* as one of the six drug-resistant microbes to which new therapies are urgently needed (Pitout and Laupland, 2008).

So far, no study has been conducted on ESBL production on both *E. coli* and *K. pneumoniae* simultaneously in Ethiopia. This study is aimed to determine prevalence and antibiotic susceptibility pattern of ESBL producing *E. coli* and *K. pneumoniae* from inpatients and outpatients that attend Jimma University Specialized Hospital. It also identifies possible risk factors for infections with ESBL producing *E. coli* and *K. pneumoniae*.

METHODS AND MATERIALS

Laboratory based comparative cross-sectional study design was conducted from September 2011 to February 2012 at Jimma University specialized hospital (JUSH), Ethiopia. The hospital is a 300 bedded teaching hospital which covers population of over 1 million. Sample size was estimated using Epi-info statistical software package (version 3.4.3, WHO Atlanta) for cross sectional studies of two population proportion to attain inpatient to outpatient ratio of 2:1. Patients' demographic data, clinical diagnoses, risk factor and specimen types were recorded for all patients included during the study period by using a questionnaire. All collected specimens were inoculated on the MacConkey agar (Oxoid, England). *E. coli* and *K. pneumoniae* was identified by their characteristic colony appearance: pink or yellow to colorless colonies (due to fermentation of lactose) from MacConkey agar, Gram-staining reaction and confirmed by the pattern of biochemical profiles using standard procedures (Koneman et al., 2006). An isolate was considered as *E. coli* when it is Indole positive, citrate negative, lysine positive, gas and acid producer, ferments mannitol, urea negative and motile. An isolate was identified as *K. pneumoniae* when it is indole negative, citrate positive, ferments mannitol, lysine positive, urea slow producing and non-motile. The

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Abbreviations: BSI, Blood stream infection; **CLSI,** Clinical and Laboratory Standards Institute; **CTX-M,** Cefotaximase-Munich; **DDST,** double disk synergy test; **ESBL,** extended spectrum β-lactamase; **ESBL-EK,** ESBL-producing *Escherichia coli* and *K. pneumoniae*; **ESBL-EC,** ESBL-producing *E. coli*; **ESBL-Kp,** ESBL-producing *K. pneumoniae*; **MDR,** multi drug resistant; **PBP,** penicillin-binding protein; **SHV,** sulphydryl variable; **TEM,** for Temoniera-name of a patient; **TMP,** trimethoprim; **SMX,** sulfamethoxazole; **WHO,** World Health Organization.

antimicrobial susceptibility was done by using Kirby-Bauer disc diffusion technique on Mueller Hinton agar (Oxoid, England) with commercially available antimicrobial discs. Strains were tested against the following antimicrobial agents: cefotaxime (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), amoxicillin-clavulanic acid (20/10 µg), cephalothin (30 µg), ampicillin (10 µg), carbenicillin (100 µg), trimethoprim-sulfamethoxazole (25 µg), chloramphenicol (30 µg), norfloxacin (10 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), nalidixic acid (30 µg), gentamicin (10 µg), amikacin (30 µg), and tetracycline (30 µg).

All *E. coli* and *K. pneumoniae* isolates were screened for ESBL production by disk diffusion method using ceftazidime (30 µg), cefotaxime (30 µg) and ceftriaxone (30 µg) antibiotic disks (Oxoid & MAST) as recommended by Clinical and Laboratory Standards Institute (CLSI, 2005). Each disc was placed on Muller Hinton agar manually and incubated for 16-18 hours at 35°C. Isolates with reduced susceptibilities to cefotaxime (zone diameter of ≤27 mm), ceftazidime (zone diameter of ≤22 mm) and/or ceftriaxon (zone diameter of ≤25 mm) was suspected as a potential ESBL producer (Clinical and Laboratory Standard Institute, 2005). Potential ESBL producers were confirmed by double disc synergy test (DDST). A susceptibility disc containing amoxicillin-clavulanate was placed in the centre of a Mueller-Hinton agar plate, and a disc containing 30µg of ceftazidime, ceftriaxone and cefotaxime were placed 15 mm (centre to centre) from the amoxicillin-clavulanate disc. Discs were incubated at 37°C for 16-18 h. Enhancement of inhibition zone of any these of cephalosporin discs on the side facing the amoxicillin-clavulanate disc was interpreted as ESBL positive (Jarlier et al., 1988). Multidrug resistance was defined as resistance to 2 or more classes of antibiotics (quinolones, trimethoprim-sulfamethoxazole, tetracycline or aminoglycosides).

The data was processed and analyzed for descriptive statistics using SPSS statistical software, version 16.0. All variables were examined by univariate analysis using the Chi-square or Fisher's exact test, as appropriate. Multivariate analysis was performed for variables that were independently associated with ESBL-infection on univariate analysis. *P*-value less than 0.05 was considered statistically significant. The study was done after gaining a full approval from the Ethical Review Board of Jimma University and Jimma University Specialized Hospital.

RESULTS

Patient population and source of specimens

Overall, 471 patients were included in the study (314 inpatients and 157 outpatients). From these, 273 (58%) were females and 198 (42%) were males. The mean age of participants was 31.15 years (±16.97 SD). *E. coli* and *K. pneumoniae* were isolated from 112 (23.8%) clinical specimens, constituting 85 (18%) and 27 (5.7%) of total prevalence, respectively. These isolates were recovered from urine 46(40.2%), vaginal swab 25(22.3%), sputum 18(17%), pus 14(12.5%), eye discharge 6(5.4%) and blood 3(2.7%). Three-fourth (n=84) of isolates were obtained from inpatients and the remaining one-fourth (n=28) was from outpatients. ESBL producing *E. coli* and *K. pneumoniae* was detected in 43/112 (38.4%) of the isolates. The mean age of patients infected by ESBL producers was 36.79 years (±18.89 SD). The majority 31/43 (72.1%) of ESBL isolates were obtained from females and the rest 12/43 (27.9%) were isolated from males. There was no association between ESBL production and specific sex groups (p > 0.05). Nineteen (70.4%) isolates of *K. pneumoniae* was found to be positive for ESBL. ESBL production was significantly higher among *K. pneumoniae* than *E. coli* isolate (p < 0.01). The prevalence of ESBL-producing *E. coli* and *K pneumonia* was 4/157 (2.5%) in outpatients and 39/314 (12.4%) in inpatients, and thus the risk of development of ESBL-production was 5 times higher in inpatients as compared to outpatients with significant difference (p < 0.05) (Table 1).

Table 1. Distribution of ESBL-production according to isolates and settings.

	Total isolate N (%)	ESBL Producers	Non ESBL producers	*P-value*
Organism				
K. pneumoniae	27(24.1)	19(70.4)	8(11.6)	<.01
E coli	85(75.9)	24(28.2)	61(88.4)	
Department				
Inpatient	84(75)	39(46.4)	45(53.6)	.002
Out patient	28(25)	4(14.3)	24(85.7)	

Associated factors for ESBL-EK

All included variables were evaluated among inpatients and only five variables were analyzed among outpatients. On univariate analysis, prior exposure to antibiotic was the associated with ESBL-production among both hospitalized and non-hospitalized patients. Treatment with third generation cephalosporins, severity of illness, length of hospital stay and chronic heart failure (CHF) and medical ward admission were additionally associated with ESBL infection among hospitalized patients. On multivariate analysis, treatment with third generation cephalosporin (ceftriaxone) is the only risk factor associated with ESBL infection (Table 2a and b).

Antibiotic resistance profile of ESBL-EK

The *ESBL* producing *E. coli* and *K. pneumoniae* were significantly resistant to third-generation cephalosporins as compared to non-producers (p<0.05) (Table 3). Resistance conferred by ESBL producing *K. pneumoniae* and *E. coli* to ceftazidime, cefotaxime and ceftriaxone was 97.7, 100 and 100%, respectively. On the other hand, non ESBL isolates were almost susceptible to third generation cephalosporins with 91.3, 98.6 and 100% susceptibility against ceftazidime, cefotaxime and ceftriaxone, respectively. Good susceptibility was observed with amikacin in both ESBL (83.7%) and non ESBL producers ((97.1%). Both ESBL producer and non-producer isolates were completely (100%) resistant to carbenicillin.

Table 2a. Characteristics of ESBL-EK and non-ESBL-EK infected patients among outpatient settings.

Characteristic	Category	ESBL positive	ESBL negative	*P-value* (Univariate)
Out-patient variable				
Sex	Female=21	2	19	0.*212*
	Male=7	2	5	
Previous antibiotic medication	Yes=14	4	10	0.031
	No=14	0	14	
Previous hospital admission	Yes=3	1	2	0.318
	No=25	3	22	
History of ICU admission	Yes=0	0	0	
	No=28	4	24	-------
Severity of illness	Critical=8	2	6	*0.305*
	Subcritical=20	2	18	
Recent surgery	Yes=2	1	1	0.134
	No=26	3	23	

Table 2b. Characteristics of ESBL-EK and non-ESBL-EK infected patients among inpatient settings.

Characteristic	Category	ESBL positive	ESBL negative	*P-value* (Univariate)	AOR (C.I 95%)
In-patient variable					
Sex	Female=64	29	35	0.458	
	Male=20	10	10		----------
Previous antibiotic medication	Yes =60	32	28	0.045	0.350(0.11-1.111)
	No =24	7	17		
Previous hospital admission	Yes=16	7	9	0.811	
	No=68	32	36		----------
Length of hospital stay	>15 days=29	19	10	0.011	0.470(0.167-1.319)
	<15 days=55	20	35		
Treatment with 3G cephalosporins	Yes=27	21	6	<0.001	0.141(0.046-0.431)
	No=57	18	39		
History of ICU admission	Yes=5	3	2	0.530	
	No=79	36	43		----------
IV insertion	Yes=62	31	31	0.271	
	No=22	8	14		----------
Severity of illness	Critical	37	34		
	Subcritical	2	11	0.015	1.035(0.157-6.805)
Recent surgery	Yes =16	4	12	0.056	
	No=68	35	33		----------
Underlying disease	Diabetes	0	2	0.183	
	Cardiac failure	13	5	0.013	
	Hypertension	1	3	0.379	
	Hepatitis	2	0	0.124	----------
	None	23	35	1	
Type of ward	Medical=44	25	19	0.045	
	Surgical=12	7	5	0.372	
	Gynecology 20	3	17	1	----------
	Pediatrics =4	3	1	0.24	

Table 3. Comparison of the susceptibility profiles of ESBL-producing and non–ESBL-producing EK.

Antibiotic	Total isolate N (%)		ESBL-EK (n=43) N (%)		Non ESBL-E (n=69) N (%)		P- value
	R	S	R	S	R	S	
Ceftazidime	48(42.9)	64(57.1)	42(97.7)	1(2.3)	6(8.7)	63(91.3)	<0.01
Cefotaxime	44(39.3)	68(60.7)	43(100)	0	1(1.4)	68(98.6)	<0.01
Ceftriaxon	43(38.4)	69(61.6)	43(100)	0	0	69(100)	<0.01
AMC	56(50)	56(50)	38(88.4)	5(11.6)	18(26.1)	51(73.9)	<0.01
Cephalothin	97(86.6)	15(13.4)	43(100)	0	54(78.3)	15(21.7)	0.001
Gentamicin	42(37.5)	70(62.5)	36(83.7)	7(16.3)	6(8.7)	63(91.3)	<0.01
Amikacin	9(8)	103(92)	7(16.3)	36(83.7)	2(2.9)	67(97.1)	0.011
Nalidixic acid	59(52.7)	53(47.3)	36(83.7)	7(16.3)	23(33.3)	46(66.7)	<0.01
Ciprofloxacin	48(42.9)	64(57.1)	33(76.7)	10(23.3)	15(21.7)	54(78.3)	<0.01
Norfloxacin	43(38.4)	68(60.7)	29(67.4)	14(32.6)	14(20.3)	55(79.7)	<0.01
Nitrofurantoin	31(27.7)	81(72.3)	22(51.2)	21(48.8)	9(13)	60(87)	<0.01
Chloramphenicol	49(43.8)	63(56.2)	33(76.7)	10(23.3)	16(23.2)	53(76.8)	<0.01
Tetracycline	79(70.5)	33(29.5)	39(90.7)	4(9.3)	40(58)	29(42)	<0.01
TS	77(68.8)	35(31.2)	41(95.3)	2(4.7)	36(52.2)	33(47.8)	<0.01
Ampicillin	93(83)	19(17)	43(100)	0	50(72.5)	19(27.5)	<0.01
Carbenicillin	112(100)	0	43(100)	0	69(100)	0	-

AMC-amoxacillin/clavulinate, TS- trimethoprim/sulfamethoxazole.

Table 4. Resistance to specific antimicrobials in isolates from inpatients versus outpatients.

Antibiotic	Susceptibility profile n (%)				P- value
	Inpatient		Outpatient		
	R	S	R	S	
Ceftazidime	43(51.2)	41(48.8)	5(17.9)	23(82.1)	0.002
Cefotaxime	39(46.4)	45(53.6)	5(17.9)	23(82.1)	0.007
Ceftriaxon	39(46.4)	45(53.6)	4(14.3)	24(85.7)	0.003
AMC	47(56)	37(44)	9(32.1)	19(67.9)	0.029
Cephalothin	76(90.5)	8(9.5)	21(75)	7(25)	0.037
Gentamicin	38(45.2)	46(54.8)	4(14.3)	24(85.7)	0.003
Amikacin	9(10.7)	75(89.3)	0	28(100)	0.071
Nalidixic acid	49(58.3)	35(41.7)	10(35.7)	18(64.3)	0.038
Ciprofloxacin	41(48.8)	43(51.2)	7(25)	21(75)	0.027
Norfloxacin	37(44)	47(56)	6(21.4)	22(78.6)	0.033
Nitrofurantoin	28(33.3)	56(66.7)	3(10.7)	25(89.3)	0.020
Chloramphenicol	41(48.8)	43(51.2)	8(28.6)	20(71.4)	0.062
Tetracycline	64(76.2)	20(23.8)	15(53.6)	13(46.4)	0.022
TS	63(75)	21(25)	14(50)	14(50)	0.013
Ampicillin	73(86.9)	11(13.1)	20(71.4)	8(28.6)	0.059
Carbenicillin	84(100)	0	28(100)	0	___

Resistance pattern between outpatient and inpatient isolates

Generally, inpatient isolates showed higher rates of resistance to most tested antibiotics, when compared with outpatient isolates. The difference in susceptibility between inpatient and outpatient isolates was statistically significant for 12 (75%) of the 16 tested antibiotics ($p<0.05$). However, the rates of resistance to amikacin, chloramphenicol, ampicillin and carbenicillin, were not significantly different between inpatient and outpatient isolates (Table 4).

Multi drug resistant ESBL-EK

The resistance rates of ESBL isolates to 2 or more classes

Table 5. The resistance rates of ESBL isolates to 2 or more classes of non-beta lactam antibiotics.

Antibiotic combination	Resistance rate N (%)
TS and T	38 (88.4)
TS, T and NA	35 (81.4)
TS, T, NA and CN	30 (70)
TS, T, NA, CN and CIP	26 (60.5)
TS, T, NA, CN, CIP and CAF	22 (51.2)
TS, T, NA, CN, CIP, CAF and F	9 (20.9)
TS, T, NA, CN, CIP, CAF, F and Ak	3 (7)

T- Tetracyclin, TS- trimethoprim-sufamethoxazole, NA- nalidixic acid, CN- gentamicin, CIP- ciprofloxacin, C- chloramphenicol, F- nitrofurantoin, Ak- amikacin.

classes of antibiotics are given in Table 5, descending from the lowest to the highest resistant isolates. ESBL-EK generally showed higher rates of resistance to antibiotics tested than non-producers. About 88.4% of ESBL isolate were multi drug resistant exhibiting cross-resistance against both cotrimoxazole and tetracycline. Resistance to three non beta-lactam antibiotics was observed among 35 (81.4%) isolates; in addition approximately 70% of ESBL positive isolates were cross-resistant to four non beta-lactam antibiotics (tetracycline, cotrimoxazole, nalidixic acid and gentamicin). The coexistence of ESBL phenotypes with five, six and seven types of non beta-lactam antibiotics were 26 (60.5%), 22 (51.2%) and 9 (20.9%) respectively. Three (7%) ESBL isolates were completely resistant to all panels of antibiotics tested.

DISCUSSION

ESBLs are widespread all over the world. The prevalence and genotype of ESBLs from clinical isolates vary according to the country and even hospital at which they are isolated from (Kim et al., 2010). The overall prevalence of ESBLs in the current study was 38.4% (43/112). This frequency is higher than continental surveys conducted in Europe (11%), South America (18.1%), North America (7.5%) and Asia-Pacific (14.2%) regions (Hawser et al., 2011; Turner, 2005). The higher prevalence seen in our study as compared to developed countries might be explained by the fact that developed countries have strict infection control policies and practices, shorter average hospital stays, better nursing barriers that are known to substantially decrease the chances of acquisition and spread of ESBL producing strains.

On the other hand, the prevalence of ESBL observed in this study is lower than that of a study done in Tanzania (45.2%) conducted variably on urinary isolates (Moyo et al., 2010). The decline observed in our study can be attributed to the inclusion of various types of specimens. Regardless of such myriad variation, this finding agrees with previous reports on ESBL production done in United Arab Emirates (Al-Zarouni et al., 2008).

Although *E. coli* ranks higher in the number of infection occurrences than *K. pneumoniae*, the predominant ESBL producer in our setting is *K. pneumoniae*. ESBL production was significantly higher among *K. pneumoniae* than *E. coli* ($p<0.01$). This finding is in agreement with previous report done among *K. pneumoniae* and *E. coli* with respective prevalence of 70 and 28% in Pakistan, and 51.5 and 39.1% in Tanzania, which demonstrated predominance of ESBL production by *K. pneumoniae* than *E. coli* (Shah et al., 2003; Moyo et al., 2010). Other study had also demonstrated conquest of ESBL producing *K. pneumoniae* not only over *E. coli* but also over other group of Gram negative bacilli including the family *Enterobacteriaceae* (Galas et al., 2008). The predilection of ESBL production by *K. pneumoniae* has never been clearly explained (Mshana et al., 2009). Our observation that *K. pneumoniae* was significantly associated with ESBL production merely reflects local and worldwide epidemiology which clearly shows that ESBL production has been more frequently observed in these bacteria than in *E. coli*.

Undesirable turn of events transpired when ESBL producing *E. coli* were detected in the community. Three (75%) of the four ESBL producers from outpatients were *E. coli.* The occurrence of ESBL-producing *E. coli* isolates in the community is in keeping with the global trend of emergence of community-acquired infections caused by ESBL-producing strains, in particular those which harbor the CTX-M gene. These gene have been reported in Africa (Kariuki et al., 2007). A recent report from Japan showed that patients with fecal carriers of ESBL-producing *E. coli* contributed substantially to urinary tract infections (Niki et al., 2011). This tendency could markedly change the approach to the treatment of urinary tract infections and as well as other infections due to ESBL producing *E. coli* that are encountered in the outpatient setting.

The interesting point of the present study was a correlation between multiple antibacterial resistances and ESBL positive phenotypes. This finding indicates that ESBL-producing strains of *K pneumoniae* and *E. coli* are more likely to have diminished susceptibility to non-β-lactam antibiotics when compared with non-ESBL-producing isolates, further curtailing the number of drugs useful against these bacteria. This result has been confirmed by others (Moyo et al., 2010; Mshana et al., 2009). This is mainly associated with unique property of the large ESBL plasmid which is capable of incorporating and subsequently coding for resistant determinants to non beta-lactam antimicrobial agents (Jacoby and Sutton, 1991). Thus our study results well support the fact that ESBL producers not only confer high levels of resistance to third generation cephalosporins but also to non-beta lactams like aminoglycosides, fluoroquinolones, tetracyclines and cotrimoxazole.

Thirty-eight (88.8%) of ESBL isolates showed multi drug resistance from 2 to 8 types of non beta lactam antibiotics tested. Of particular concern is that three (7%) of ESBL producing isolates were resistant to all panels of antibiotics used. Thus, the presence of an ESBL is a good marker of the MDR phenotype. In the present study, amikacin has retained good susceptibility rates due to its absence of use as empirical therapy and nonexistence of considerable cross-resistance with third generation cephalosporins. Similarly, study from Egypt also showed the high percentage of susceptibility to amikacin among antibiotics tested (Zaki, 2007). These findings have significant implication for empirical management of patients infected with ESBL organisms using amikacin.

Third-generation cephalosporin specifically ceftriaxone is one of the most commonly used classes of antibiotics for hospitalized patients in Ethiopia, as observed during this study, exerting predominant selective pressure for the emergence of resistance among pathogenic microorganisms. On multivariable analysis, use of third generation cephalosporins was identified as the only risk factors significantly associated with infection due to ESBL producers. This finding is in accordance with previous studies disclosing that indiscriminate use of third-generation cephalosporins was related to the selection of ESBL-producing organisms (Lautenbach et al., 2001). Use of cephalosporins is not only associated with ESBL infection, but also it was found to be a risk factor for colonization with ESBL producing organisms (Levy et al., 2010). As a result, the higher percentage of ESBL-producing *E. coli* or *K. pneumoniae* in the current study may be due to the greater selective pressure imposed by extensive use of third-generation cephalosporins. This association has been best displayed by interventional study which demonstrated decline in the prevalence of ESBL-EK colonization from 7.9 to 5.7% following restriction of third-generation cephalosporins (Bisson et al., 2002). In general, the association of ESBL with third-generation cephalosporins suggests that the best way to control these pathogens in our hospital is to reduce the use of these antibiotics.

ESBLs occurrence was significantly higher among isolates from inpatients than outpatients [39 (46.4%) vs. 4(14.3%)] (P =0.002). Nosocomial acquisition of ESBL producing *E. coli* and *K. pneumoniae* bacteremia has been reported indicating that hospital environment played a crucial role in maintenance of ESBL producing organism (Kang et al., 2006). Furthermore higher rate of fecal carriage of ESBL-producing organisms among inpatients (26.1%) than among outpatients (15.4%) is documented elsewhere in Saudi Arabia (Kader et al., 2007). This suggests that nosocomial acquired organisms are more likely to become ESBL producer.

More than 70% of strains isolated from both inpatient and outpatient groups showed resistance to ampicillin, cephalothin and carbenicillin. This may alarm the presence of the classic beta lactamase which was recognized among this isolates prior to isolation of ESBL enzymes (Livermore, 1995). In addition, marked resistance to tetracycline and co-trimoxazole was observed in the inpatient group (77.4% to tetracycline and 75% to TMP-SMZ) and with slight decrease in the outpatient group (51.7% to tetracycline and 48.3% to TMP-SMZ), this may be explained by the frequent use of both antibiotics in the community as well as in our hospital. Therefore, the use of this drug is questionable in suspected *E. coli* and *K. pneumoniae* infection in our setting.

Limitation

We are familiar with the limitation of study, as noted in all observational studies. Molecular epidemiological study and characterization of ESBL types were not conducted. Second, we did not assess certain clinical features such as ICU admission and urinary catheterization as potential risk factor for infection with ESBL producing EK due to little number of cases which are insignificant number to be included during study period. Third, our study was conducted in Jimma University Specialized Hospital, and the results may not be generalizable with other institutions.

Conclusion

Our data provide evidence that the ESBL is prevalent in Jimma University Specialized Hospital. Majority of ESBL producing strains are from inpatients and only few are community isolates. Therefore, it is very urgent to address the problem of hospital acquired infections caused by ESBL-producing bacteria. Use of third generation cephalosporin was the only independent predictor of ESBL-producing *E. coli* or *K. pneumoniae* infection. These agents should not be used in infections due to confirmed ESBL producers because resistance to third-generation cephalosporin is often accompanied by resistance to fluoroquinolones, aminoglycosides, TMP-SMX and tetracyclines.

Conflict of interest

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are grateful to Jimma University Faculty of Medical Science, Department of Medical Laboratory Science and Pathology and all staff of Microbiology and Parasitology Teaching Laboratory of the department. The staffs of Jimma University Specialized Hospital are acknowledged for their support in facilitating specimen collection and patient information.

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