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Prevalence of extended spectrum βeta-lactamase producing *Escherichia coli* from patients diagnosed with urinary tract infections in Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State.

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Abstract

Background: Extended spectrum Beta-lactamases (ESβLs) are variants of beta lactamase enzymes that are capable of hydrolyzing broader spectrum of beta-lactams antibiotics. The enzymes have mutation in the gene at the active site that is believed to be the cause of high Beta lactamase activity. ESβL mediate resistance to all third generation cephalosporins, including monobactams. This study was carried out to determine the prevalence of ESBL producing *Escherichia coli* from patients presenting with cases of urinary tract infection at Olabisi Onabanjo University Teaching Hospital between April and June 2016.

Method: Urine samples from cases of UTI were centrifuged and the subnatants were diluted serially up to 10^{-5} with sterile distilled water. A loopful of each of the last two dilutions was streaked on a plate of sterile Eosin Methylene Blue (EMB) agar. The plates were incubated at 37°C for 24 hrs. Plates that elicited growth were sub-cultured and stored for further use. Gram staining and conventional biochemical tests including indole, citrate utilization, hydrogen sulphide utilization, nitrate, catalase and urease tests were conducted on selected distinct colonies with green metallic sheen on the EMB culture plate. Antimicrobial susceptibility was determined by disc-diffusion method. ESBL detection was done by using the double-disc synergy test. An antibiotic disc of amoxicillin-clavulanic acid (Oxoid, UK) was placed at the center of the plate and discs containing Ceftazidime (CAZ - 30µg) (Oxoid, UK), Ceftriaxone (CRO - 30µg) Aztreonam (ATM - 30µg) were sited 0.2cm equidistant from the amoxicillin-clavulanic acid disc. After aerobic incubation at 37°C for 18 hours, a clear extension of the edge of the growth inhibition zone of the cephalosporins towards amoxicillin-clavulanic acid disc was measured and used as positive index of ESβL production.

Results: Of the 100 urine samples examined, 79 (79%) isolates of *Escherichia coli* were detected by conventional biochemical tests of which 30 (38%) isolates were found to exhibit ESβL production. The antibiotic susceptibility profile of the isolates elicited highest susceptibility to ofloxacin (90%), gentamicin (87%) and amoxicillin-clavulanate (53%). A progressive decrease in sensitivity to cefixime (60%) and cefuroxime (27%) – a cephalosporinase effect was recorded.

Conclusion: Judicious use of antibiotics is more important to prevent infections by these resistant organisms in the community coupled with awareness by microbiologists and clinicians serving the community as key to early detection and appropriate treatment of patients affected by ESβL producing *Escherichia coli.*

Keywords: *Urinary tract infection, ESâL Escherichia coli*

Résumé

Contexte: Les bêta-lactamases à spectre étendu (ESBL) sont des variantes des enzymes bêta-lactamase qui sont capables d'hydrolyser un plus large spectre d'antibiotiques bêta-lactames. Les enzymes ont une mutation dans le gène sur le site actif qui est considéré comme la cause d'une activité bêta-lactamase élevée. L'ESβL opère une résistance à toutes les céphalosporines de troisième génération, y compris les mono-bactames. Cette étude a été réalisée pour déterminer la prévalence d'Escherichia coli produisant des ESβL chez des patients présentant des cas d'infection urinaire à l'Hôpital d'Enseignement Universitaire Olabisi Onabanjo entre avril et juin 2016.

Méthode: Des échantillons d'urine provenant de cas d'UTI ont été centrifugés et les sous-produits ont été dilués en série jusqu'à 10^{-5} avec de l'eau distillée stérile. Une boucle de chacune des deux dernières dilutions a été striée sur une plaque d'agar stérile Éosine Méthylène Blue (EMB). Les plaques ont été incubées à 37 ° C pendant 24 heures. Les plaques qui ont suscité une croissance ont été sous-cultivées

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et stockées pour une utilisation ultérieure. La coloration à Gram et les tests biochimiques classiques, y compris l'utilisation d'indole, de citrate, d'utilisation de sulfure d'hydrogène, d'essais de nitrate, de catalase et d'uease ont été réalisés sur des colonies différentes avec un brillant métallique vert sur la plaque de culture EMB. La susceptibilité aux antimicrobiens a été déterminée par la méthode de diffusion de disque. La détection ESβL a été effectuée en utilisant le test de synergie à double disque. Un disque antibiotique d'amoxicilline-acide clavulanique (Oxoid, UK) a été placé au centre de la plaque et des disques contenant du Ceftazidime (CAZ-30μg) (Oxoid, UK), Ceftriaxone (CRO - 30μg) Aztreonam (ATM - 30μg) ont été installés 0,2 cm équidistant du disque acide amoxicilline-clavulanique. Après une incubation aérobique à 37 ° C pendant 18 heures, on a mesuré un prolongement clair du bord de la zone d'inhibition de la croissance des céphalosporines par rapport au disque acide amoxicilline-clavulanique et utilisé comme indice positif de la production ESβL.
Résultats: Sur les 100 échantillons d'urine examinés, 79 (79%) des isolats d'Escherichia coli ont été détectés par des tests biochimiques classiques dont 30 (38%) isolés ont été exposés à la production ESBL. Le profil de sensibilité aux antibiotiques des isolats a suscité la plus forte susceptibilité à l'ofloxacine (90%), à la gentamicine (87%) et à l'amoxicilline-clavulanate (53%). Une diminution progressive de la sensibilité au cefixime (60%) et à la cefuroxime (27%) - un effet céphalosporinase a été enregistré.
Conclusion: l'utilisation judicieuse des antibiotiques est plus importante pour prévenir les infections par ces organismes résistants dans la communauté, associée à la prise de conscience par les microbiologistes et les cliniciens qui servent la communauté comme élément clé du dépistage précoce et du traitement approprié des patients atteints d'ESβL produisant *Escherichia coli*.

Mots-clés: *Infection des voies urinaires, ESβL, Escherichia coli*

Introduction

Urinary tract infection (UTI) is a common problem diagnosed and treated in urgent care medical practice. An estimated eight million episodes of UTI occur in the United State of America each year with one out of three women requiring treatment for UTI before age 24 years of age. There is no statistical data or accurate facts on the numbers of infected individuals in Nigeria. This could be due to the self-management of infections as a result of high cost of medical care in the country. Urinalysis including Gram staining and culture may assist with diagnosis, but also add to the cost of care. UTIs can affect the lower urinary tract (cystitis) or upper tract (pyelonephritis) [1].

Varieties of antibiotics are available for treating UTIs, but changing antibiotic sensitivities make appropriate empiric treatment a moving target over time. Antibiotic chemotherapy for UTIs has been found to have a profound effect on the urethral microbiota, for example, 4-6 weeks after the administration of amoxicillin or bacampicillin, the urethral microbiota were found to be dominated by *Escherichia coli* and *Staphylococcus epidermidis* respectively [2]. The urethral microbiota of healthy individuals is dominated by lactobacilli, and these organisms are important in preventing UTIs. Administration of amoxicillin or bacampicillin, therefore, results in urethral communities that would have a reduced ability to prevent subsequent colonization by uropathogens and possible re-infection [2].

UTIs which occur in men, pregnant women, and patients with immunosuppression or urinary tract abnormalities, such as congenital malformations, urinary calculi, recent urologic instrumentation, indwelling catheters, neurogenic bladder and kidney transplant, are considered complicated and require more complex decision-making [2].

There is an increase of antibiotic resistance in bacteria that cause either community infections or hospital acquired infections [3]. Of particular interest are the multidrug resistant pathogens, e.g. *Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus*. Administration of co-trimoxazole appears to have little effect on the urethral microbiota. *Pseudomonas aeruginosa,* an organism often detected in the urethra, may be isolated from the urethra of individuals with recurrent UTIs that are frequently treated with antibiotics [3].

Extended-spectrum β-lactamases (ESβLs) are enzymes produced by many Gram-negative bacteria which have ability to change the susceptibility of different antibiotics. They are plasmid-mediated enzymes with the capability to hydrolyze and inactivate a broad spectrum β-Lactam antimicrobials, including third-generation cephalosporins, penicillins and aztreonam but their action is inhibited by clavulanic acid [4].

ESβLs are usually inhibited by β-lactamase-inhibitors such as clavulanic acid and tazobactam, which makes a difference between ESBL- and Amp-C (aminopenicillin hydrolysing cephalosporinase)-β-lactamases producing bacteria. ESβLs have been widely reported in several Gram-negative bacteria,

but they are usually linked to the family Enterobacteriaceae, including *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp. and *Escherichia coli*. The increase of ESβL producing *E. coli* among humans is worrisome since their mechanism of resistance is involved in the failure of the pharmacological treatment of diseases. Majority of community-acquired ESβL-producing *Escherichia coli* infections present as urinary tract infections (UTIs), sometimes complicated by pyelonephritis or bacteraemia [5].

Previous use of antibiotics (especially fluoroquinolones) has been constantly identified as an independent risk factor for urinary infections. Treatment options may be limited depending on the degree of multidrug resistance. Frequently, ESβL-producing *Escherichia coli* exhibits co-resistance to antibiotic agents such as trimethoprim-sulfamethoxazole, ciprofloxacin and gentamicin. A major complicating factor is the possibility of horizontal gene transfer, which can disseminate resistance to multiple antibiotics in a single step [6]. Thus, few treatment options remain. Fosfomycin or amoxicillin/clavulanate may be the treatment options for UTI, although not all ESβL producing organisms are susceptible. On the other hand, carbapenems are considered the first line agents for more severe infections like pyelonephritis and bacteremia caused by ESβL producers, given the data on favourable clinical outcome with this class. Though still rare, severe sepsis has been reported with community-acquired ESβL-producing *Escherichia coli*. Of concern is horizontal transmission of ESβL - producing *Escherichia coli* from a mother to a newborn causing bacteremia which has also been reported. The progressive reduction in the efficacy of ESβL antibiotics in the family of cephalosporins as a result of evolution of resistance is becoming alarming and this must be taken into consideration in the management of infections caused by ESβL producing *Escherichia coli*.. Therefore, knowledge of the local epidemiology and risk factors for these infections is crucial in choosing appropriate empiric therapy for severe *Escherichia coli* infections that originate in the community [7].

This study was an attempt to evaluate the phenotypic prevalence of ESβL from isolates of *Escherichia coli* obtained from urinary tract infection patients and assess their antimicrobial resistance profiles.

Materials and methods

Collection of samples

Mid-stream urine samples voided into clean sterile specimen bottles were obtained from patients visiting the outpatient department of the Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun state, Nigeria.

Isolation and Identification *Esch. coli*

Urine samples from cases of UTI were centrifuged and the subnatants were diluted serially up to 10^{-5} with sterile distilled water. A loopful of each of the last two dilutions was streaked on a plate of sterile Eosin Methylene Blue (EMB) agar. The plates were incubated at 37°C for 24 hrs. Plates that elicited growth were sub-cultured on agar slants and stored for further use.

Gram staining and conventional biochemical tests including indole, citrate utilization, hydrogen sulphide utilization, nitrate, catalase and urease tests were conducted on selected distinct colonies with green metallic sheen to confirm the presence of *Escherichia coli*.

Antibiogram

Antimicrobial susceptibility was determined using disc diffusion method. The confirmed *Escherichia coli* isolates were sub-cultured into 5ml sterile nutrient broth and incubated overnight. Three-fold serial dilution of the overnight culture which was then adjusted to 0.5 McFarland standard (10^8CFU/ml) was streaked on Mueller Hinton Agar. The following antibiotic discs for Gram negative bacteria were used; Ceftazidime (30µg), Cefuroxime (30µg), Gentamicin (10µg), Cefixime (5µg), Ofloxacin (5µg), Amoxicillin/clauvulanic (30µg), Nitrofurantoin (300µg) and Ciprofloxacin (5µg). The discs were placed firmly on the inoculated culture plates. The plates were incubated at 37°C for 24 hours. The diameter of zones of growth inhibition was measured in millimeter and was interpreted as resistant or sensitive using CLSI standard (2014).

Detection of extended spectrum β- lactamase (ESβL)

ESBL detection was done using the double-disc synergy test. This was done on all the isolates which was sub-cultured into 5ml sterile nutrient broth and incubated overnight. A disc impregnated with amoxicillin-clavulanic acid (AMC – 20/10 µg) (Oxoid, UK) was placed at the center of the culture plate and discs containing ceftazidime (CAZ - 30µg) (Oxoid,UK), ceftriaxone (CRX - 30µg) and aztreonam (ATM - 30µg) were sited 0.2cm equidistant from the amoxicillin-clavulanic acid disc. After aerobic incubation at 37°C for 18 hours, a clear extension of the edge of the growth inhibition zone of cephalosporins towards amoxicillin-clavulanic acid disc was measured as positive index of ESBL production.

Results

In this study, 100 samples of urine were collected from 100 patients (40 male and 60 female). Of the total number of samples examined, seventy-nine(79) isolates of *Escherichia coli* were obtained, The gender distribution of the isolates was twenty nine 29(72.5%) from males and fifty 50 (83.3%) from females. The highest prevalence of UTI was found within the 31—40 years age range with prevalence of (85%).However, the variation observed in the prevalence of the infection in both sexes was not statistically significant (p>0.005).(Table 1.0)

Table 1: Age and Gender Distribution of the UTI isolates.

	Urinary Tract Infection		
Age (in years)	Number Examined	Number Positive	(%) Positive
0-10	5	3	60
11-20	15	10	66.7
21-30	20	15	75
31-40	20	17	85
41-50	20	18	90
51-60	10	8	40
61-70	10	8	40
Gender			
Male	40	29	72.5
Female	60	50	83.3
Total	100	79	

Tables 2 and 3 show the susceptibility pattern and numbers that were positive for ESBL tests. Thirty (30) out of the 79 E*sherichia coli* isolates were positive for ESβL. The total percentage was found to be the highest in ceftazidime (93%) resistance while resistance gentamicin and ofloxacin were recorded to tables 4 and 5 which show the numbers and percentage of the isolated strains that were resistant to two or more different classes of antibiotics. The multiple antibiotic resistance index (MARI) of varied values were recorded. MARI of 46.67% were recorded for 14 numbers of *Esch. Coli* while isolate *E24*with (MARI) 0.75 was the only one with multiple resistance to six antibiotics.

Plate 1.0: is a selected culture plate of the pictorial representation of the ESβL producing *Escherichia coli* patterns of growth towards amoxicillin-clavulanate placed at the center of culture plate.

Discussion

The gender distribution of the isolates was found to be the highest in female (50) in comparison to male (29), which could be attributed to the closeness of female urethra to the anus, sexual activities associated with the use of diaphragm and hygiene practice which can vary from one individual to another. The age gender distribution of the samples collected within the period of two months (April to May, 2016) shows variation in percentage which could be due to underlying factors associated with the immune status of the visiting patients at that very period coupled with self-management with un-prescribed herbal formulae. The prevalence of 38% of the extended spectrum beta lactamase *Esch. coli* were obtained from the total numbers of patients that visited the teaching hospital within the period of two months and can be said to be significant with the reports of the trend of the gradual upsurge of ESβ L *Escherichia coli* in many information compendia and are mostly community acquired [8].

A total percentage (93%) of the isolates studied were resistant to ceftazidime, 53% to cefuroxime and 30% to cefixime. This can be suggestive of the decreasing potency of these ESBL antibiotics on the spectra of *Esch. coli* obtained from UTI cases. This portends an impending danger in the management of ESβL associated with the infection. The resistance of 60% and 53% recorded against ciprofloxacin and amoxicillin-clavulanic respectively could be attributed to strain differentiations and probably frequent exposure of these drugs (at a lesser concentration) in the management of UTI infection, which is corroborated with the findings of Ellner (1987) on the epidemiological factors affecting the antimicrobial resistance of common bacterial isolates [9].

The susceptibility of the isolates to ofloxacin (90%), gentamicin (87%) and cefixime (60%) invitrobially could be attributed to the potency of the concentration of these antibiotics on the isolates, which could make them to be considered drugs of choice in the treatment of ESβL *Esch. coli* associated urinary tract infection. Nitrofurantoin was recorded to be totally ineffective on any of the isolates tested, an indication of absolute resistance of the isolates to this amino-furan derivative antibiotics.

The multiple antibiotic resistance index obtained in this study is 46.7% against 14 isolates could be due to many factors such as genetic make-up, selective pressure from the environment and abuse of antibiotic use in the management of UTI infection, which buttressed the findings of David (2005) on rational antibiotic treatment of outpatient genitourinary infection in a changing environment [10].

Table 2: Antibiogram and ESBL determination of isolate of *Escherchia coli* isolate

Isolates Codes	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CPR	ESBL
	S≥21 R≤ 15 I = 18-20	S≥23 R≤ 14 I= 15-22	S≥15 R≤ 12 I= 13-15	S≥ 19 R≤ 15 I= 16-18	S≥ 16 R≤ 12 I=13-15	S≥ 18 R≤13 I=14-17	S≥17 R≤ 14 I= 15-16	S≥ 17 R≤15 I=16-20	
E1	10 R	26 S	24S	22 S	26 S	10R	0R	38S	+
E3	0 R	30 S	18 S	28 S	23 S	0 R	0 R	34 S	+
E7	0 R	30 S	10 R	30 S	26 S	40 S	0 R	30 S	+
E9	0 R	0 R	24 S	12 R	24 S	16 I	0 R	24 S	+
E10	0 R	0 R	17 S	20 S	21 S	0 R	0 R	30 S	+
E12	0 R	0 R	22 S	20 S	30 S	21 S	0 R	0 R	+
E13	10 R	18 I	18 S	30 S	28 S	12 R	0 R	0 R	+
E17	12 R	0 R	26 S	20 S	27 S	25 S	0 R	0 R	+
E19	10 R	0 R	24 S	10 R	36 S	27 S	0 R	10 R	+
E21	0 R	0 R	22 S	12 R	26 S	36 S	0 R	30 S	+
E23	0 R	18 I	14 I	30 S	32 S	27 S	0 R	0 R	+
E24	0R	20 I	0 R	10 R	40 S	0 R	0 R	10 R	+
E27	10 R	0 R	20 S	20 S	27 S	36 S	0 R	0 R	+
E28	0 R	0 R	16 S	32 S	26 S	36 S	0 R	20 I	+
E35	10 R	0 R	14 I	36 S	12 R	18 S	0 R	10 R	+
E38	0 R	0 R	20 R	16 I	25 S	0 R	0 R	0 R	+
E41	0 R	0 R	28 S	24 S	38 S	25 S	10 R	0 R	+
E42	0 R	16 I	24 S	27 S	31 S	28 S	10 R	0 R	+
E45	0 R	18 I	26 S	22 S	32 S	27 S	0 R	0 R	+
E49	0 R	12 R	30 S	10 R	30 S	18 S	0 R	20 I	+
E50	15 R	0 R	18 S	10 R	30 S	21 S	0 R	20 I	+
E52	20 I	0R	16 S	27 S	26 S	36 S	0 R	10 R	+
E54	10 R	0 R	22 S	28 S	27 S	15 I	0 R	15 R	+
E55	10 R	0 R	26 S	30 S	30 S	17 I	0 R	0 R	+
E60	0 R	24 S	22 S	20 S	0 R	0 R	0 R	10 R	+
E62	19 I	26 S	16 S	16 I	16 S	12 R	0 R	0 R	+
E68	0 R	32 S	16 S	14 R	18 S	12 R	0 R	40 S	+
E70	0 R	28 S	16 S	12 R	16 S	18 S	0 R	30 S	+
E75	12 R	24 S	20 S	10 R	20 S	0 R	0 R	21 S	+
E79	0 R	20 I	22 S	16 I	14 I	12 R	0 R	0 R	+

Table 3: Percentage (susceptibility/resistance) profiles of the isolates of *Escherichia coli* exposed to selected antibiotics.

Antibiotics	% Resistance	% Susceptibility	% Intermediate
Ceftazidime	93%	-	7%
Cefuroxime	53%	27%	20%
Gentamicin	7%	87%	7%
Cefixime	30%	60%	10%
Ofloxacin	7%	90%	3%
Amoxicillin-Clavulanate	37%	53%	10%
Nitrofurantoin	100%	-	-
Ciprofloxacin	60%	30%	10%

Table 4: Multiple antibiotic resistance (MAR) index and resistance pattern of isolated *Escherichia coli*

Isolate Code	Resistance pattern	MAR index	Resistance category
E1	CAZ, AUG, NIT	0.375	MDR
E3	CAZ, AUG, NIT	0.375	MDR
E7	CAZ, GEN, NIT	0.375	MDR
E9	CAZ, CRX, CXM, NIT	0.5	MDR
E10	CAZ, CRX, AUG, NIT	0.5	MDR
E12	CAZ, CRX, NIT, CPR	0.5	MDR
E13	CAZ, AUG, NIT, CPR	0.5	MDR
E17	CAZ, CRX, NIT, CPR	0.5	MDR
E19	CAZ, CRX, CXM, NIT, CPR	0.625	MDR
E21	CAZ, CRX, CXM, NIT	0.5	MDR
E23	CAZ, NIT, CPR	0.375	MDR
E24	CAZ, GEN, CXM, AUG, NIT, CPR	0.75	MDR
E27	CAZ, CRX, NIT, CPR	0.5	MDR
E28	CAZ, CRX, NIT	0.375	MDR
E35	CAZ, CRX, OFL, NIT, CPR	0.625	MDR
E38	CAZ, CRX, AUG, NIT, CPR	0.625	MDR
E41	CAZ, CRX, NIT, CPR	0.5	MDR
E42	CAZ, NIT, CPR	0.375	MDR
E45	CAZ, NIT, CPR	0.375	MDR
E49	CAZ, CXM, NIT	0.375	MDR
E50	CAZ, CRX, CXM, NIT	0.5	MDR
E52	CRX, NIT, CPR	0.375	MDR
E54	CAZ, CRX, NIT, CPR	0.5	MDR
E55	CAZ, CRX, NIT, CPR	0.5	MDR
E60	CAZ OFL, AUG, NIT, CPR	0.625	MDR
E62	AUG, NIT, CPR	0.375	MDR
E68	CAZ, CXM, AUG, NIT	0.5	MDR
E70	CAZ, CXM, NIT	0.375	MDR
E75	CAZ, CXM, AUG, NIT	0.5	MDR
E79	CAZ, AUG, NIT, CPR	0.5	MDR

MAR= Number of antibiotic resistance ÷ Number of antibiotics used

Table 5: Percentage Multiple Antibiotic Resistance Index of *Escherichia coli* isolates

MAR Index	No. of *Escherichia coli* isolates	Percentage (%)
0.375	11	36.67
0.5	14	46.67
0.625	4	13.33
0.75	1	3.33
Total	30	100%

Percentage MARI = MARI ÷ Total number of isolates multiplied by 100

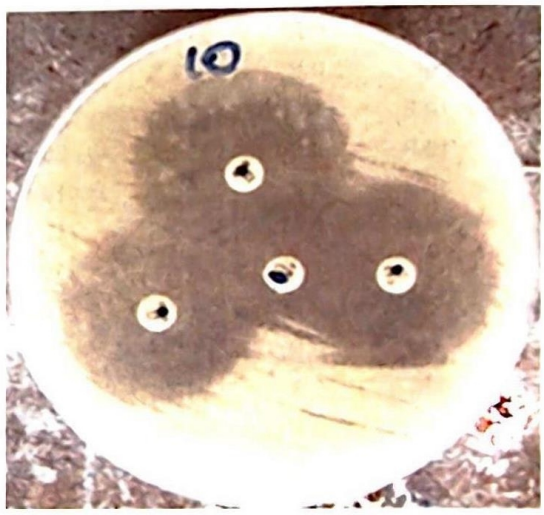

Fig. 1: Pictorial representation of ESβLproducing isolates of *Escherichia coli*

The recent global spread of ESBL -producing *E. coli* in the community has been explosive. They are likely already part of the flora in communities worldwide, making elimination impossible, just as we have seen with community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) [11].

Judicious use of antibiotics is highly imperative to prevent infections by these resistant organisms in the community. On the local level, awareness by microbiologists and clinicians serving the community is key to early detection and appropriate treatment of patients affected by ESāL producing *Escherichia coli.* [12]. Rational use of antibiotics should be encouraged. Antibiotics should

not be available for sale without prescription from a physician or a health care professional; also patients to whom antibiotics are prescribed should be monitored closely to ensure that they adhere strictly to the dosage regimen.

References

1. David RD. DeBlieux PM and Press R. Rational antibiotic treatment of outpatient genitourinary infections in a changing environment. Am J Med. 2005; 118(Suppl) 7A:7S-13S.
2. Little P, Turner S, Rumsby K, *et al.* Developing clinical rules to predict urinary tract infection in primary care settings: sensitivity and specificity of near patient tests (dipsticks) and clinical scores. Br J Gen Pract. 2006 Aug; 56 (529): 606–612.
3. Bent S, Nallamothu BK, Simel DL, *et al.* Does this woman have an acute uncomplicated urinary tract infection? JAMA. 2002;287:2701-2710.
4 Bradford, P.A. Extended spectrum â-lactamases in the 21stcentury: characterization, epidemiology and detection of this important resistance threat. Clin. Microbiol. Rev. 2001: 14, 933–951.
5. Pitout J.D.D. Infections with extended-spectru â-lactamase-producing Enterobacteriaceae: changing epidemiologyand drug treatment choices. (2010):- Drugs, 70: 313–333.
6. Coque T.M., Baquero F. and Canton R. Increasing prevalence of ESâL-producing Enterobacteriaceae in Europe. (2008):Euro Surveillance, 13(47), pii=19044.
7. Randall L.P., Clouting C., Horton R.A., *et al.* Prevalence of Escherichia coli carrying extended-spectrum â-lactamases (CTX-M and TEM-52) from broiler chickens and turkeysin Great Britain between 2006 and 2009. (2011): Journal of Antimicrobial Chemotherapy, 66: 86–95
8. Bonnet, R.. Growing group of extended-spectrum â-lactamases:the CTX-M enzymes. Antimicrob. Agents Chemother. 2004: 48(1),1-14
9. Bortolaia V., Larsen J., Damborg P. and Guardabassi L. Potential pathogenicity and host range of extended-spectrum-lactamase-producing Escherichia coli isolates from healthy poultry. (2011): Applied Environmental Microbiology. 77: 5830–5833.
10. El-khizzi,N.A. and Bakheshwain S.M. Prevalence of extended spectrum beta-lactamases among Enterobacteriaceae isolated fromblood culture in atetiary care hospital.2006:.Saudi Medical journal. 27;37-40
11. Ellner,P.D.,D.J.Fink,H.C.Neu, and M.F.Parry. Epidemiological factors affecting antimicrobial resistance of common bacterialisolates. 1987: J.Clin. Microbiol. 25:1668–1674.
12. David RD, DeBlieux PM and Press R. Rational antibiotic treatment of outpatient genitourinary infections in a changing environment.2005: Am J Med.118 (Suppl)7A:7S.