Phenotypic expression and prevalence of multi drug resistant extended spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* in Karachi, Pakistan

Sakina Fatima[1], Iyad Naeem Muhammad[1], Muhammad Naseem Khan[2] and Subia Jamil[3]
[1]Department of Pharmaceutics, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan
[2]Microbiological Analytical Centre, FMRRC, PCSIR Labs. Complex, Karachi, Pakistan
[3]Department of Pharmacology, Faculty of Pharmacy, Jinnah University for Women, Karachi, Pakistan

Abstract: The aim of the present study was to evaluate the prevalence rate of ESBL producing Gram negative isolates of *E. coli*, *K. pneumoniae* and *P. mirabilis*, to determine the association of various factors with ESBL production and therapeutic options for the treatment. Total 352 isolates were subjected for identification of ESBL by double disc synergy test. Antimicrobial susceptibility was performed using CLSI guidelines and statistical association between ESBL/Non ESBL producers were determined by chi square at significant level of 0.05. A total of 96 isolates were ESBL positive (27%), females were 67% whereas males were 33%. *E. coli* was most prevalent pathogen (82%) followed by *Klebsiella pneumoniae* (17%). Furthermore 75% of ESBL associated infections were urinary tract infections. 95% of ESBL producing isolates were multidrug resistant and tazobactam/piperacillin combination and imipenem are good choices with 100% and 97% susceptibility respectively. *E coli* (OR 2.83, 95% CI 1.585-5.072, RR 2.22, *p* 0.0004) and *K. pneumoniae* (OR 0.52, 95% CI 0.285-0.952, RR 0.609, *p* 0.032) were significantly associated with ESBL production. The spread of ESBL producing multidrug resistant *E. coli* and *K. pneumoniae* has increased and proper screening for ESBL identification is needed because of limited therapeutic antibiotic choices.

Keywords: ESBL, *Escherichia coli, Klebsiella pneumoniae,* double disc synergy test, multidrug resistance.

INTRODUCTION

Infections associated with Gram negative bacteria producing Extended Spectrum Beta Lactamase (ESBL) is a major risk emerged all over the world for health care concerns. Treatment options for infections due to these multi-drug resistant ESBL producing organisms are therefore limited, and initial empirical therapy is often ineffective resulting in increased mortality (Anderson *et al*., 2006; Schwaber & Carmeli, 2007; Tumbarello *et al*., 2007).

Global epidemiological studies indicate the prevalence of multi-drug resistant (MDR) ESBL producing *Enterobacteriaceae* both in the community acquired and healthcare facility associated infection (Ma *et al*., 2015) (Park *et al*., 2012) (Raji *et al*., 2015) (Wegner *et al*., 2013). In *Enterobacteriaceae E Coli* and *Klebsiella* are dominant pathogens as ESBL producers (Fatemeh *et al*., 2012; Jacoby & Munoz-Price 2005)

ESBL related community acquired infections increased because of *bla* $_{CTX-M}$ gene which originated from environmental bacteria and transferred to a plasmid which is highly transmissible, this link is related in circulation of ESBLs in the community (Pitout, Nordmann, Laupland, & Poirel, 2005). The CTX-M ESBLs in *E. coli* have been isolated from domestic animals, food products, sewage, and stool samples from healthy individuals. *E coli* has replaced *Klebsiella* species as the predominant species of ESBL-producing Enterobacteriaceae in the much of world (Carattoli *et al*., 2005; Kojima *et al*., 2005) In Pakistan, recent studies indicated increased prevalence of ESBL in *E Coli* (Ali *et al*., 2016; Rahman *et al*., 2016).

The objective of the present study was to evaluate the prevalence of ESBL production among Gram negative isolates of *E. coli*, *K. pneumoniae* and *P. mirabilis*, their susceptibility and resistance pattern and statistical association between ESBL and non-ESBL producing isolates.

MATERIALS AND METHODS

Sample collection

A total of 1005 clinical isolates of *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *P. mirabilis* form various specimen (Urine, Pus, stool, Sputum, Throat swab, HVS, Ascitic Fluid, Cystic fluid, semen) collected over a period of one year from diagnostic laboratories. Antimicrobial susceptibilities were determined for initial screening of ESBL, double disc synergy test was performed for ESBL identification and ESBL associated risk factors were also calculated.

Antimicrobial susceptibility test

For antimicrobial susceptibility test, disc diffusion method was used on Mueller Hinton Agar. The antibiotics

**Corresponding author:* e-mail: iyadnaeem@uok.edu.pk

tested were: Amikacin, Gentamicin, Tobramycin, Ampicillin, Amoxicillin/Clavulanic acid, Imipenem, Tazobactum/Piperacillin, Vancomycin, Cefuroxime, Cefixime, Cefotaxime, Ceftazidime, Ceftriaxone, Fosfomycin, Nalidixic Acid, Urixin, Ofloxacin, Enoxacin, Ciprofloxacin, Sparfloxacin, Doxycyclin, and Trimethoprim/Sulphamethoxazole. Resistance and susceptibilities were determined by zones of inhibition recommended in guidelines provided by CLSI.

Inclusion criteria for isolates

Out of 1005 clinical isolates, 352 non-repeated were selected after initial screening of ESBL, for phenotypic confirmation test. Inclusion criteria for ESBL initial screening in study isolates were, determination of zones of inhibition by disc diffusion method including ceftazidime ≤22 mm, cefotaxime ≤27mm and ceftriaxone ≤25mm for *E. coli* and *K. pneumonia*; for *P. mirabilis* ceftazidime≤22 mm and cefotaxime ≤27 mm.

Double disc synergy test for identification of ESBL

The presence of ESBL was performed by using antibiotic discs of Cefotaxime (30µg), Ceftazidime (30µg) and Amoxicillin/ Clavulanic acid (20/10µg). In the middle, amoxicillin/clavulanic acid were placed and the other two were placed 30mm apart from middle, and were incubated for 24 hours. Enhanced zone of inhibition of cephalosporin discs(s) towards amoxicillin/clavulanic indicated synergy with clavulanic acid and ESBL presence (Jarlier *et al*., 1988). For positive and negative controls of ESBL producers, *Klebsiella pneumoniae* (ATCC 700603) and *Escherichia coli* (ATCC 25922) were used.

STATISTICAL ANALYSIS

Pearson's Chi-Square test was used for various study factors for determination of association between ESBL and non-ESBL producers, p value <0.05 was considered as significant. The odds ratio (OR) with 95% confidence interval (CI) and relative risk (RR) were determined. Factors were age (<50 years), gender (female), microorganism (*E. coli* and *K. pneumoniae*) and specimen (UTI). ESBL associated antibiotic resistance for Amikacin, Imipenem, Fosfomycin, Cephalosporin and fluoroquinolones was also determined. Statistical analysis was performed using SPSS (version 20).

RESULTS

ESBL producing isolates and demographics

Out of 352 isolates, 96 were ESBL positive (27.2%), 62 (67%) were from females and 32 (33%) from males. *E. coli* was the most prevalent microorganism, 79 were ESBL positive (82.29%), *K. pneumoniae* and *P. mirabilis* were 16 (17%) and 1 (1%), respectively. Statistical association of various factors were tested by Chi Square test listed in table 4. Significant results were found with *E. coli* and *K. pneumoniae* related infections (OR 2.83, 95% CI 1.585-5.072, RR 2.22, *p* 0.0004 and OR 0.52, 95% CI 0.285-0.952, RR 0.609, *p* 0.032, respectively). Among total 96 ESBL producing isolates, 72 (75%) were isolated from urine specimen. table 1 depicts the distribution of ESBL producing microorganism from various specimen collected. Other factors such as gender, age and specimen were non-significant p>0.05 with ESBL production (table 4)

Antimicrobial susceptibility

All ESBL producing isolates were sensitive to TZP i.e., 100% susceptible and IMP, AK, FOS were 95.83%, 92.7 % and 90.62%, respectively. Cephalosporin and fluoroquinolones were highly resistant antibiotic classes listed in table 3. In antibiotic resistance amoxicillin/clavulanic acid was significant with ESBL positive isolates (OR 0.45, 95% CI 0.2794 -0.726, RR 0.67, *p* 0.001) while other antibiotics were non-significant (table 4).

Multi drug resistance

Microorganisms resistant to at least one antibiotic in three antibiotic classes considered as multi drug resistant (Magiorakos *et al*., 2012) 95% were multidrug-resistant while only 5% were non-MDR i.e. having resistance against less than 3 antibiotic classes. In *E. coli*, *K. pneumoniae* and *P. mirabilis*, non-MDR were 2.53%, 12.5 and 100%, respectively and MDR were 97.46%, 87.5% and 0%, respectively.

DISCUSSION

Increased resistance with production of extended spectrum β-lactamase (ESBL) enzyme in Gram negative bacteria of family *Enterobacteriaceae* with emerged multi drug resistance is a widespread issue for health care setups. WHO has reported antimicrobial resistance of ESBL producing *E. coli* to third generation cephalosporin and fluoroquinolones, *K. pneumoniae* to third generation cephalosporin and carbapenems as an international concern (WHO 2014). The objective of this study was to determine the prevalence of ESBL producing Gram negative clinical isolates including *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *P. mirabilis*, their antimicrobial resistance and risk association with ESBL production.

In the present study, double disc synergy test was used, which determined 96 isolates from a total of 352 i.e., 27% of isolates were ESBL positive. The most prevalent pathogens associated with ESBL production was *E. coli* (n=79) followed by *K. pneumoniae* and *P. mirabilis* were 16 and 1, respectively. The study revealed an increased prevalence in females (n=64) i.e., 67% and 33% were in males (n=32), but it was found statistically not significant

Table 1: Specimen distribution of ESBL producing isolates

Specimen	No. of Isolates	%age	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*
Urine	72	75	63	9	-
Pus	9	9.375	8	1	-
Sputum	3	3.57143	-	3	-
Stool	4	4.16667	4	-	-
HVS	5	5.20833	3	2	-
Throat Swab	1	1.04167	-	-	1
Cystic Fluid	1	1.04167	1	-	-
Semen	1	1.04167	-	1	-

*HVS: high vaginal swab

Table 2: Antimicrobial susceptibility profile of ESBL producing isolates (n=96)

Antibiotics used	Susceptible Isolates (%)	Resistant Isolates (%)
AK (Amikacin)	89 (93)	7 (7)
CN (Gentamicin)	62 (65)	34 (35)
NN (Tobramycin)	61 (64)	35 (36)
AML (Ampicillin)	3 (3)	93 (97)
AMC (Amoxicillin/Clavulanic acid)	56 (58)	40 (42)
IPM (Imipenem)	92 (96)	4 (4)
TZP (Tazobactam/Piperacillin)	96 (100)	0 (0)
V (Vancomycin)	15 (16)	81 (84)
CXM (Cefuroxime)	38 (40)	58 (60)
CFM (Cefixime)	36 (38)	60 (62)
CTX (Cefotaxime)	46 (48)	50 (52)
CRO (Ceftriaxone)	48 (50)	48 (50)
CAZ (Ceftazidime)	48(50)	48 (50)
FOS (Fosfomycin)	87 (91)	9 (9)
NA (Nalidixic Acid)	13 (14)	83 (86)
UR (Urixin)	12 (13)	84 (87)
OFL (Ofloxacin)	40 (42)	56 (58)
ENX (Enoxacin)	43 (45)	53 (55)
CIP (Ciprofloxacin)	39 (41)	57 (59)
SPX (Sparfloxacin)	43 (45)	53 (55)
DOX (Doxycycline)	2 (2)	94 (98)
SXT (Trimepthoprim/Sulphamethoxazole)	31(32)	65 (68)

(p=0.908) for ESBL production (table 4). Previous studies found preponderance in females (Kiratisin *et al*., 2008) and female gender was not at risk with (p=0.089) and statistically insignificant (Kateregga *et al*., 2015). A total of 72 isolates were recovered from Urine and 75% of infections associated with urinary tract infections, a preponderance was found in ESBL associated UTI but was statistically non-significant (table 4). Increased prevalence of ESBL positive UTI has also been reported earlier (Kateregga *et al*., 2015)

Among *E. coli* isolated, 82% were found ESBL positive and a significant relation (p=0.0004) was found with more than two times risk in *E. coli* for the production of ESBL (table 4). In a previous study from Pakistan, high prevalence of ESBL in *E. coli* isolated has been reported with 48% patients between 50-60 years of age (Shah *et al*., 2002) Another study reported 41% of ESBL positive *E. coli* (Jabeen *et al*., 2005). A high prevalence of ESBL positive *E. coli* associated UTI was detected in females i.e. 70.3% (n=45) (fig. 2), however the difference was not statistically significant. Ali and coworkers have reported high prevalence of UPEC in females with 40% being ESBL positive (Ali *et al*., 2016). Out of 96 ESBL positive isolates 17% were *K pneumoniae* with a less prevalence rate in our region, previous studies indicated more prevalent ESBL-producing *K pneumoniae* and the percentages were 44.3% (Shaikh *et al*., 2015) and 72.7% (Kateregga *et al*., 2015). *K. pneumoniae* was also significantly associated with the ESBL production (p=0.032), Kateregga and co-workers reported statistical insignificant results in *E coli* and *K pneumoniae* in Uganda.

Table 3: MDR and Non MDR Distribution of the isolates

Microorganism	Non MDR (%)	MDR (%)
E Coli (n=79)	2 (2.53)	77 (97.46)
Klebsiella (n=16)	2 (12.5)	14 (87.5)
Proteus (n=1)	1 (100)	0
Total (n=96)	5 (5.20)	91 (94.79)

Table 4: Association between ESBL production in UTI, Gender, age, *E coli*, *K pneumoniae* and antimicrobial resistance

Factors	Non ESBL n=256 (%)	ESBL n=96 (%)	Odds ratio (CI 95 %)	p value	RR
UTI	180 (70)	72 (75)	1.26 (0.720 -2.240)	0.754	1.06
Female	169 (66)	64 (67)	1.03 (0.626 -1.692)	0.908	1.02
<50 yrs	139 (54)	56 (58)	1.17 (0.733 -1.894)	0.496	1.12
E coli	159 (62)	79 (82)	2.83 (1.585 - 5.072)	0.0004*	2.22
K pneumoniae	71 (28)	16 (17)	0.52 (0.285 - 0.952)	0.032*	0.609
Antibiotics resistance					
AK	18 (7)	7 (7)	1.04 (0.42- 2.574)	0.9325	1.03
IMP	20 (8)	4 (4)	0.51 (0.171- 1.512)	0.2345	0.53
FOS	35 (13)	9 (9)	0.65 (0.301- 1.416)	0.2805	0.68
CTX	143(56)	50 (52)	0.85 (0.536-1.3747)	0.526	0.93
CRO	139(54)	48 (50)	0.84 (0.5263-1.3462)	0.471	0.92
OFL	160(62)	56 (58)	1.19 (0.7380-1.9204)	0.478	0.93
CIP	95 (37)	39 (40)	1.15 (0.717-1.8735)	0.5453	1.09
AMC	157 (61)	40 (41)	0.45 (0.2794-0.726)	0.0011*	0.67

CI= Confidence Interval
RR=Relative Risk *P<0.05= Significant value

Fluoroquinolones and cephalosporin are highly prescribed antibiotic classes. An increased rate of resistance against third generation cephalosporin and fluoroquinolones was found in our study (table 2). Previous studies have also reported high fluoroquinolone resistance against community acquired or healthcare associated urinary tract and intra-abdominal infections, exceeding 50% in some regions of the world, mostly in Asia (Dalhoff 2012) Resistance to ciprofloxacin has also increased in last 10 years (Guyomard-Rabenirina *et al*., 2016) In Karachi, Pakistan, in last 20 years fluoroquinolones resistance has increased gradually. It was 14% in 1997 (Sturm *et al*., 1997) and raised up to 35% in 2004 (Gul *et al*., 2004). (Ali *et al*., 2016) reported 60% resistance in ESBL associated *E. coli* with Ciprofloxacin and extended spectrum cephalosporin, which is similar to the results of our study in terms of resistance i.e. 59% and 50-62% resistance to ciprofloxacin and cephalosporin, respectively, in ESBL producers. ESBL producing Gram negative isolates were found 100% and 96% susceptible to Piperacillin/Tazobactam and Imipenem, respectively. Hence, both are good choices for treatment of infections associated with ESBL production previously reported (Falagas and Karageorgopoulos, 2009; Gavin *et al*., 2006; Shaikh *et al*., 2015).

On the basis of antimicrobial susceptibility profiles, 95% of isolates were multi drug resistant. Increased rates of multi drug resistance in *E. coli* (97.46%) and *K. pneumoniae* (87.5%) were found, even higher percentages than previously reported i.e. 70.6% in *E. coli* (Azap *et al*., 2010). Amoxicillin/clavulanic acid is a β-lactamase antibiotic and a good choice against ESBL associated urinary tract infections in case of fluoroquinolones resistance (Paterson and Bonomo 2005). (Rodríguez-Baño *et al*., 2008) reported 29% resistance of Amoxicillin/ Clavulanic acid in ESBL producers while in the present study 42% resistance has been recorded and a significant relationship was revealed (p=0.001) between amoxicillin/ clavulanic acid resistance and ESBL production, thus not recommended for treatment of quinolones resistant ESBL associated urinary tract infection isolates in our region.

CONCLUSION

The widespread of multi drug resistant ESBL-producing *E coli* and *K pneumoniae* has increased in Karachi, Pakistan. Our study suggested tazobactam/piperacillin and Imipenem as treatment choices. To improve therapeutic outcomes and decreased mortality rate clinical laboratories need proper screening of ESBL positive isolates in their culture reports. The use of third generation Cephalosporin and fluoroquinolones must be restricted and must only be used in the light of susceptibility patterns.

ACKNOWLEDGMENT

Department of Pharmaceutics, Faculty of Pharmacy, University of Karachi for research facility and Dr. Essa Laboratories, Microbiology department for the collection of clinical isolates.

REFERENCES

Ali I, Rafaque Z, Ahmed S, Malik S and Dasti JI (2016). Prevalence of multi-drug resistant uropathogenic Escherichia coli in Potohar region of Pakistan. *Asian. Pac. J. Trop. Biomed.,* **6**(1): 60-66.

Anderson DJ, Engemann JJ, Harrell LJ, Carmeli Y, Reller LB and Kaye KS (2006). Predictors of mortality in patients with bloodstream infection due to ceftazidime-resistant Klebsiella pneumoniae. *Antimicrob. Agents Chemother.,* **50**(5): 1715-1720.

Azap Ö, Arslan H, Şerefhanoğlu K, Çolakoğlu Ş, Erdoğan H, Timurkaynak F and Senger S (2010). Risk factors for extended spectrum β-lactamase positivity in uropathogenic Escherichia coli isolated from community acquired urinary tract infections. *Clin. Microbiol. Infect.,* **16**(2): 147-151.

Carattoli A, Lovari S, Franco A, Cordaro G, Di Matteo P and Battisti A (2005). Extended-spectrum β-lactamases in Escherichia coli isolated from dogs and cats in Rome, Italy, from 2001 to 2003. *Antimicrob. Agents. Chemother.,* **49**(2): 833-835.

Clinical and Laboratory Standards Institute (CLSI) (2014). Performance standards for antimicrobial susceptibility testing. *CLSI Document M100-S24.* Wayne, PA, USA.

Dalhoff A (2012). Global fluoroquinolone resistance epidemiology and implictions for clinical use. *Interdiscip Perspect Infect Dis,* Article ID 976273: 37.

Falagas M and Karageorgopoulos D E (2009). Extended-spectrum β-lactamase-producing organisms. *J. Hosp. Infect.,* **73**(4): 345-354.

Fatemeh A, Emran A, Elnaz K, Mohammad J and Mahboubeh N (2012). The frequency of extended spectrum beta lactamase (ESBL) in *Escherichia coli* and *Klebsiella pneumonia*: A report from Mashhad. *Iran. J. Med. Bacteriol.,* **1**(3): 12-19.

Gavin PJ, Suseno MT, Thomson RB, Gaydos JM, Pierson CL, Halstead DC and Brossette SE (2006). Clinical correlation of the CLSI susceptibility breakpoint for piperacillin-tazobactam against extended-spectrum-β-lactamase-producing Escherichia coli and Klebsiella species. *Antimicrob. Agents. Chemother.,* **50**(6): 2244-2247.

Gul N, Mujahid TY and Ahmad S (2004). Isolation, Identification and Antibiotic Resistance Profile of Indigenous. *Pak. J. Biol. Sci.,* **7**(12): 2051-2054.

Guyomard-Rabenirina S, Malespine J, Ducat C, Sadikalay S, Falord M, Harrois D and Talarmin A (2016). Temporal trends and risks factors for antimicrobial resistant Enterobacteriaceae urinary isolates from outpatients in Guadeloupe. *BMC. Microbiol.,* **16**(1): 1-8.

Jabeen K, Znfar A and Hasan R (2005). Frequency and sensitivity pattern of Extended Spectrum beta Lactamase producing isolates in a tertiary care hospital laboratory of Pakistan. *J. Pak. Med. Assoc.,* **55**(10): 436.

Jacoby G A and Munoz-Price L S (2005). The New β-Lactamases. *N. Eng. J. Med.,* **352**(4): 380-391.

Jarlier V, Nicolas MH, Fournier G and Philippon A (1988). Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: Hospital prevalence and susceptibility patterns. *Rev. Infect. Dis.,* **10**(4): 867-878.

Kateregga JN, Kantume R, Atuhaire C, Lubowa MN and Ndukui JG (2015). Phenotypic expression and prevalence of ESBL-producing Enterobacteriaceae in samples collected from patients in various wards of Mulago Hospital, Uganda. *BMC. Pharmacol. Toxicol.,* **16**(1): 1-6.

Kiratisin P, Apisarnthanarak A, Laesripa C and Saifon P (2008). Molecular characterization and epidemiology of extended-spectrum-β-lactamase-producing *E. coli* and *Kl. pneumoniae* isolates causing health care-associated infection in Thailand, where the CTX-M family is endemic. *Antimicrob. Agents. Chemother.,* **52**(8): 2818-2824.

Kojima A, Ishii Y, Ishihara K, Esaki H, Asai T, Oda C and Yamaguchi K (2005). Extended-spectrum-β-lactamase-producing *E. coli* strains isolated from farm animals from 1999 to 2002: Report from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. *Antimicrob. Agents. Chemother.,* **49**(8): 3533-3537.

Ma X, Wu Y, Li L, Xu Q, Hu B, Ni Y and Robert J (2015). First multicenter study on multidrug resistant bacteria carriage in Chinese ICUs. *BMC Infectious Diseases,* **15**(1): 1-10.

Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C and Olsson Liljequist B (2012). Multidrug resistant, extensively drug resistant and pandrug resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.,* **18**(3): 268-281.

Park SH, Byun JH, Choi SM, Lee DG, Kim SH, Kwon JC and Yoo JH (2012). Molecular epidemiology of extended-spectrum β-lactamase-producing Escherichia coli in the community and hospital in Korea: emergence of ST131 producing CTX-M-15. *BMC. Infect. Dis.,* **12**(1): 149.

Paterson DL and Bonomo RA (2005). Extended-spectrum β-lactamases: A clinical update. *Clin. Microbiol. Rev.,* **18**(4): 657-686.

Pitout JD, Nordmann P, Laupland KB and Poirel L (2005). Emergence of Enterobacteriaceae producing

extended-spectrum β-lactamases (ESBLs) in the community. *J. Antimicrob. Chemother.,* **56**(1): 52-59.

Rahman H, Naeem M, Khan I, Khan J, Haroon M, Bari F and Qasim M (2016). Molecular prevalence and antibiotics resistance pattern of class A bla CTX-M-1 and bla TEM-1 beta lactamases in uropathogenic *Escherichia coli* isolates from Pakistan. *Turk. J. Med. Sci.,* **46**(3): 897-902.

Raji MA, Jamal W, Ojemeh O and Rotimi VO (2015). Sequence analysis of genes mediating extended-spectrum beta-lactamase (ESBL) production in isolates of Enterobacteriaceae in a Lagos Teaching Hospital, Nigeria. *BMC. Infect. Dis.,* **15**(1): 259.

Rodríguez-Baño J, Alcalá JC, Cisneros JM, Grill F, Oliver A, Horcajada JP and Cuenca M (2008). Community infections caused by extended-spectrum β-lactamase–producing *Escherichia coli*. *Arch. Intern. Med.,* **168**(17): 1897-1902.

Schwaber MJ and Carmeli Y (2007). Mortality and delay in effective therapy associated with extended-spectrum β-lactamase production in Enterobacteriaceae bacteraemia: A systematic review and meta-analysis. *J. Antimicrob. Chemother.,* **60**(5): 913-920.

Shah A, Hasan F, Ahmed S and Hameed A (2002). Extended-spectrum beta-lactamases in Enterobacteriaceae: Related to age and gender. *New Microbiol,* **25**(3): 363-366.

Shaikh S, Fatima J, Shakil S, Rizvi SMD and Kamal MA (2015). Risk factors for acquisition of extended spectrum beta lactamase producing *Escherichia coli* and Klebsiella pneumoniae in North-Indian hospitals. *Saudi J. Bio. Sci.,* **22**(1): 37-41.

Sturm A, Van der Pol R, Smits A, Van Hellemondt F, Mouton S, Jamil B and Sampers G (1997). Over-the-counter availability of antimicrobial agents, self-medication and patterns of resistance in Karachi, Pakistan. *J. Antimicrob. Chemother.,* **39**(4):543-547.

Tumbarello M, Sanguinetti M, Montuori E, Trecarichi EM, Posteraro B, Fiori B and Cauda R (2007). Predictors of mortality in patients with bloodstream infections caused by extended-spectrum-β-lactamase-producing Enterobacteriaceae: Importance of inadequate initial antimicrobial treatment. *Antimicrob. Agents Chemother.,* **51**(6): 1987-1994.

Wegner C, Hubner NO, Gleich S, Thalmaier U, Kruger CM and Kramer A (2013). One-day point prevalence of emerging bacterial pathogens in a nationwide sample of 62 German hospitals in 2012 and comparison with the results of the one-day point prevalence of 2010. *GMS. Hyg. Infect. Control.,* **8**(1): Doc12.

World Health Organization (2014). Antimicrobial resistance global report on surveillance summary.