https://ijrps.com

ISSN: 0975-7538
Research Article

Prevalence and antimicrobial susceptibility pattern of ESBL producing gram negative rods causing nosocomial infection

Umer Syed[1], Shakirullah[2], Aziz Ullah[3], Baharullah*[3], Hamid Iqbal[3], Abdul Wahab[4], Ali Rehman[5]

[1]Department of Microbiology, Quaid-E-Azam University, Islamabad, Pakistan
[2]Department of Microbiology, Abasyn University Peshawar, Khyber Pakhtunkhwa, Pakistan
[3]Department of Microbiology, Kohat University of Science & Technology Kohat, Khyber Pakhtunkhwa, Pakistan
[4]Department of Pharmacy, Kohat University of Science & Technology Kohat, Khyber Pakhtunkhwa, Pakistan
[5]Department of Chemistry, Kohat University of Science & Technology Kohat, Khyber Pakhtunkhwa, Pakistan

ABSTRACT

To find the possible risks to hospitalized patients from hospital environment, this study was conducted with aim to investigate about prevalence of Gram Negative Rods (GNRs), their antibiotics susceptibility profile and ESBL producing ability of strains from a tertiary care hospital in Kohat, Pakistan. Total 120 samples were collected from medical, surgical, orthopedic, children and gyneae wards. All the samples were inoculated on various media including Blood Agar, MacConky Agar, EMB Agar and Clid Agar. All GNRs were identified on the basis of biochemical tests performed on API20E kit. ESBL detection and antimicrobial susceptibility profile of all isolates was performed on Mueller-Hinton Agar plates using Kirby Bauer's Disc Diffusion method. From all the samples, *Pseudomonas aeruginosa* was isolated from 49 (40.83%) samples, *Proteus* sp. from 35 (29.17%) samples, *Klebsiella pneumoniae* from 21 (17.50%) samples and *Escherichia coli* from 15 (12.50%) samples. ESBL producing strains were found as 47.36% in *Klebsiella pneumoniae*, 46.42% in *Pseudomonas aeruginosa*, 77.7% in *E. coli*, and 20% in *Proteus* sp. Antibiotics resistance profile of both ESBL producing and non-ESBL producing bacteria was also too high.

Keywords: Gram Negative Rods; Antimicrobial; Patients

INTRODUCTION

An infection is considered as nosocomial infection by Center for the Disease Control (CDC) if it occurs due to exposure to hospital environment while according to (Qayyum *et al*. 2010) if an infection is caused at least after 48 hours of admitting a patient in a hospital is a nosocomial infection (Qayyum *et al*., 2010; Sheikh *et al.,* 2008) for which the causative agent may be a virus, bacteria or a fungi[1]. Along with patients already admitted in hospitals, healthy persons may also be affected who are in regular contact of hospital's environment (Qayyum *et al*., 2010)

Nosocomial infection was first reported in 1980s in Europe caused by bacteria especially *Enterobacteriaciae* which were producing Extended Spectrum Beta-Lactamase (ESBL) (Jain *et al*., 2003). Due to production of beta-lactamase, these strains are capable to show resistance against various antibiotics (Jones *et al*., 1998) including carbapenems, cefotaxime, ceftazidime, ceftriaxone, and monobactams (Shukla *et al*., 2004) but not cephamycins or carbapenems (Bush 2001; Bradford 2001). The β-lactamases produced by bacteria are known to protect against the cidal effect of penicillins, cephalosporins and monobactams on their cell wall synthesis. The primary mechanism responsible for the resistance to β-lactam group of antibiotics is the production and secretion of β-lactamases by the isolates of family *Enterobacteriaceae* (Sanders *et al*.,1992).This resistance is developed by a majority of gram negative bacteria using variety of resistance mechanisms (Subha *et al.,* 2002). ESBLs are mostly found in uropathogens, like *Klebsiella pneumoniae* and *E.coli.*, Other enterobacteria and to lesser extent by non-fermenting gram negative rods (Goussard *et al*., 1999; Bush *et al*., 2010)

The prominent Infections caused by beta-lactamase producing bacteria are urinary tract infections, pneumoniae, septicemia, wound infections (Khan *et al*., 2010), orthopedic infections, respiratory diseases, ecthyme gangrenosum, osteochondritis of the foot, and corneal infections (Khan *et al*., 2008). Beta lactam antibiotics are commonly used to treat infections by GNRs, but gram negative bacteria get resistant against beta lactam antibiotics by secreting a plasmid mediated enzyme called extended spectrum beta lactamase (ESBL) which were first identified in 1983 among *Klebsiella pneumoniae* and *Serratia marcescens* (Knothe *et al*., 1983; Philippon *et al*., 1989) are now produced by more than 150 bacteria (Lautenbach *et al*., 2001).

* Corresponding Author
Email: dr.baharullah@kust.edu.pk
Contact: +91-
Received on: 28-01-2013
Revised on: 20-03-2013
Accepted on: 22-03-2013

Management and the treatment of infections caused by ESBL-producers are complicated not only because of resistance to extended-spectrum cephalosporins, but also due to many ESBL genes located on large plasmids encoding resistance to many other antibiotics including aminoglycosides, chloramphenicol, sulfonamides and tetracycline antibiotics (Moland *et al*., 1998). Cross transmission of these ESBL producing bacteria in hospitalized settings have been implicated for nosocomial infections worldwide. These infections have a significant impact on patient's mortality and additional financial burden (Astagneau *et al*., 1999). This problem is increasing day by day with high ratio which is a challenging question to current clinical studies (Moland *et al*., 1998) Aim of the present study is to document the prevalence of ESBL-producing GNRs and their susceptibility to different available antibiotics and to see the antibiotics resistant pattern of nosocomial bacteria at local hospital.

MATERIALS AND METHODS

Total 120 samples were collected from medical, surgical, orthopedic, children and Gwynne wards of Liaqat Memorial Hospital (LMH) Kohat and District Headquarter Hospital (DHQ) Kohat which were studied at Microbiology Laboratory, Department of Microbiology, Kohat

Table 1: Site of Sampling

Site of Sampling	Air	Throat	Nose	Canola	Wound	Apparatus	Surface Area	Total
Number of Samples	40	20	16	08	04	06	16	120

Table 2: Samples collected from different wards

Ward	Males	Females	Air/ apparatus	Total samples
Children	04	08	06	18
Gynae	00	10	06	16
OT of Gynae	00	00	10	10
Medical	14	12	12	38
Orthopaedic	04	04	06	14
Surgical	08	04	12	24
Total samples	30	38	52	120

Table 3: Comparative resistance pattern of ESBL and non-ESBL GNR to different antibiotics

Antibiotics	*Klebsiella pneumoniae*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Proteus sp.*	
	ESBL (%)	Non ESBL (%)	ESBL (%)	Non ESBL (%)	ESBL (%)	Non ESBL (%)	ESBL (%)	Non ESBL (%)
AMC	25	33	0	0	16	71	50	0
MEM	100	100	75	0	66	60	75	50
SXT	100	33	33	0	25	33	66	50
AMP	100	100	100	0	100	100	100	100
DO	0	0	0	0	0	0	0	100
KF	100	0	0	0	100	100	0	100
CN	50	0	0	0	0	0	0	100
C	0	0	100	0	25	0	0	0
TZP	100	100	100	0	66	0	100	50

Table 4: Biochemical test results of isolated GNRs

TESTS		*E. Coli*	*K. Pneumonia*	*Proteus spp*	*P. aeruginosa*
Lactose Fermentation		+ive	+ive	-ive	-ive
Manose Fermentation		+ive	+ive	-ive	-ive
Glucose Fermentation		+ive	+ive	+ive	D
Sucrose Fermentation		D	+ive	D	-ive
Oxidase Test		-ive	-ive	-ive	+ive
Citrate Utilizing Test		-ive	+ive	+ive	+ive
Motility Test		+ive	-ive	+ive	+ive
Indole Test		+ive	-ive	-ive	-ive
Triple Sugar Iron	Slope	Y	Y	R	R
	Butt	Y	Y	Y	R
	H_2S	-ive	-ive	+ive	-ive
	Gas	+ive	+ive	+ive	-ive

D: Different species give different result, Y: Yellow, R: Red

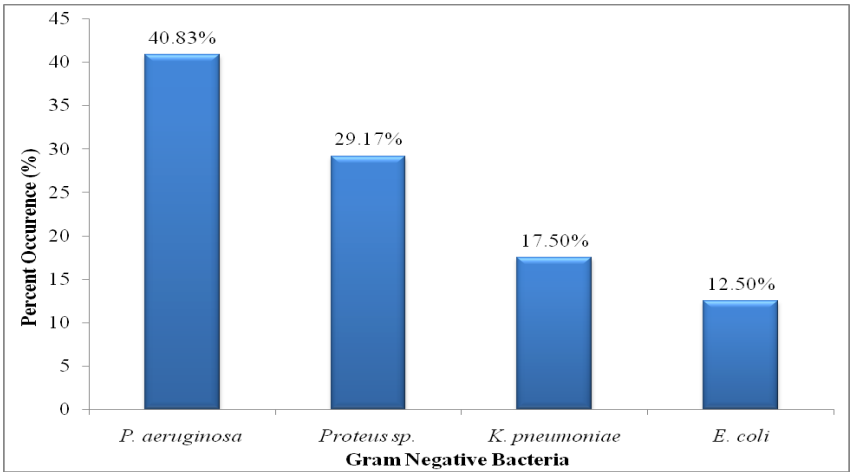

Figure 1: Percentage occurrence of different nosocomial pathogens

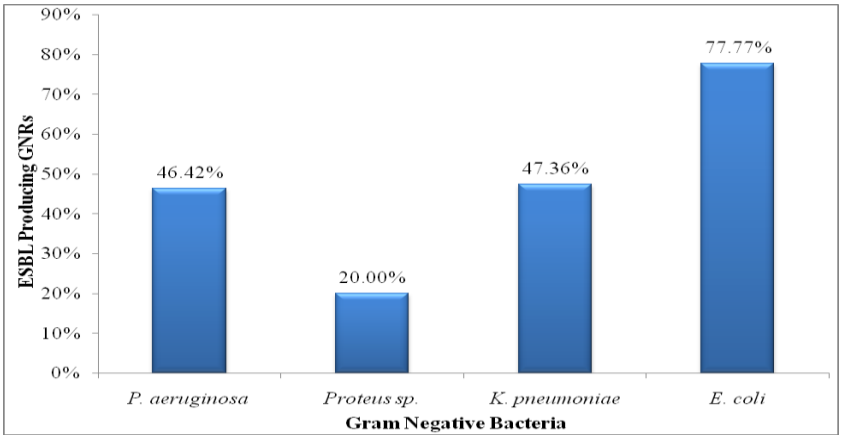

Figure 2: Percentage ESBL production by nosocomial pathogens

University of Science & Technology, Kohat (Khyber Pakhtunkhwa) Pakistan. All the samples were distributed and tabulated on the basis of type of samples collected, gender-wise and site from where the sampling was done.

These samples were cultured on MacConkey's Agar, Eosin Methylene Blue (EMB) Media and Blood Agar. Pure cultures of GNRs were isolated and purified by using pure culture technique and gram staining technique after incubation at 37°C for 24 hours. Isolated GNRs were identified by conventional biochemical tests like gram staining, Oxidase test, Indole production test, Citrate utilization test, Motility test, Lactose Fermentation test, sucrose Fermentation test, mannose Fermentation test, and Glucose Fermentation test.

ESBL detection was assessed by using Double-disk diffusion test using a disk of AMC (Amoxicillin/Clavulonic acid 20µg/10µg) placed in between CAZ (Ceftazidime 30µg) and CTX (Cefotoxime 30µg) at a distance of 20mm from each other on Mueller Hinton agar (MHA).

Antibiotic susceptibility testing was done by Kirby Bauer's Disc diffusion technique using antibiotic disk of Piperacilline/Tazobactam (100µg/10µg), Doxycycline (30µg), Ampicillin (25µg), Cephalothin (30µg), Gentamicin (10µg), Chloramphenicol (30µg), Trimethoprim/Sulfamethoxazole (5/25µg), Ciprofloxacin (5µg) and Meropenem (10µg).

RESULTS

Results from the sampling distribution showed that from total 68 out of 120 samples were from patients of different wards. In medical wards (14 Samples) and surgical wards (08 Samples) were isolated from samples collected from male patients, while overall more females (55.88%) than males (44.11%) were affected (Table 1). On the other hand, data analysis showed that most of the samples (43.33%) were belonging to air environment of the hospital (Table 2).

Different bacteria were isolated from samples included *Pseudomonas aeruginosa* with highest incidence of 40.83% followed by *Proteus sp*. (29.17%), *Klebsiella pneumoniae* (17.50%) and *Escherichia coli* with 12.50% as shown in figure 1. By using Double-disk diffusion method, ESBL production was found in all isolated gram negative rods. Figure 2 shows the percentage of ESBL producing bacteria. Highest production of ESBL was found in *E. coli* and the lowest ratio was of *Proteus sp* with 20.00% while *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were producing ESBL with rate of 47.36% and 46.42%, respectively. Isolated strains were confirmed on the basis of biochemical tests. Results of biochemical tests are shown in table 3.

Table 4 shows comparative antibiotic resistance pattern for ESBL and Non-ESBL pathogenic GNRs which was too high. Respective resistance of ESBL and Non-ESBL *Klebsiella pneumoniae* to different antibiotics was Amoxicillin/Clavulanate (25/33%), Meropenem (100%), Sulphamethoxazole/Trimethoprim (100/33%), Ampicillin (100%), Cephalothin (0%) and Gentamicin (25%). Respective resistance of ESBL and Non-ESBL *Pseudomonas aeruginosa* was Amoxicillin/Clavulanate (61%, 71%), Meropenem (66%, 60%), Sulphamethoxazole/Trimethoprim (25%, 33%), Ampicillin (100%, 100%) and Cephalothin (25%, 0%). Respective resistance of ESBL and Non-ESBL *Proteus sp*. was Amoxicillin/Clavulanate (50%, 0%), Meropenem (75%, 50%), Sulphamethoxazole/Trimethoprim (66%, 50%), Ampicillin (100%, 100%) and Cephalothin (0%, 0%).

DISCUSSION

Gram-negative bacteria are important nosocomial pathogens as Escherichia coli, *Pseudomonas aeruginosa*, *Klebsiella* sp., *Proteus* sp., and others. These organisms cause infections in hospitalized patients especially in intensive care units (ICU) and patient with malignancy, immunosuppressive disorders, burns, intravascular, central nervous system devices, mechanical ventilation and indwelling urinary catheters.

Fast spread of ESBL producing organisms, now-a-days, are creating problem to controlling infection. Proper detection of ESBL is seemed to be compulsory because susceptibility test is no longer effective.

Reports from all around the world shown that different countries have different proportion of ESBL-producing isolates (AA Shah et al., 2005) A large-scale survey of ICU in Europe found the prevalence of ESBL in *Klebsiella*. To be low (3%) in Sweden while high (34%) in Portugal and higher of (30–60%) reported in South American countries of Brazil, Venezuela and Colombia, in Japan and Australia ESBL production in *Klebsiella* was low (5%) but higher rates of 20–50% in other parts of the continent (JM Jamal et al., 2002). National Surveys of some Asian countries like Korea, Japan, Malaysia and Singapore shown an average of 5–8% of *E. coli* isolates to be ESBLs producing but also found up to 24% for other Asian countries (MT Lewis et al., 1999) Over 1-year period, 7.5% of *Enterobacteriaceae* and *Pseudomonas* isolated in Kuwait and Arabian Gulf region, were reported as being ESBL producers (W Jamal et al., 2005). In United Arab Emirates, 41% of *Enterobacteriaceae* were ESBL producers (Al- Zarouni et al., 2008). This data indicate that ESBL production varies from place to place as well as type of species that produced ESBL.

We isolate 77% ESBL-producing *E. coli* which is the highest percent ever reported in Asian countries. We found 27% *Klebsiella species* to be ESBL producing which is equal or higher reported from other Asian countries. We found 46% of *Pseudomonas aeruginosa* and 20% of the *Proteus* sp, to be ESBL producing which are much higher figures.

Other studies conducted at Pakistan detect ESBL production was 70% in *Klebsiella pneumoniae,* 28.6% in *E. coli* at Islamabad (AA Shah et al., 2004), *Escherichia coli* (45%) *Klebsiella pneumoniae* (21%), *Pseudomonas aeruginosa* (19.2%), *Enterobacter cloacae* (4.6%) and *Acinetobacter baumannii* (4.4%) at AMC Rawalpindi (AM Ali et al., 2004) and 56.9% in *E. coli*, 71.7% in *Klebsiella pneumoniae* were ESBL positive at Lahore (F Ullah et al., 2009). In neighbor countries like india ESBL production reported was 63.7% in E. coli, 14% *Klebsiella pneumoniae* in one study, other study show 70% E. coli, 13% *Klebsiella pneumoniae*, 9% *P. mirabilis* and (2%) of *Pseudomonas aeruginosa* ESBL producing (Babypadmini *et al*., 2004) while in Iran ESBL production was 12% in *Klebsiella pneumoniae,* and 21% in *E. coli*2 (Behroozi *et al*., 2010).

This data suggest that ESBL production by pathogenic bacteria varies from place to place. These also suggest that all type of pathogenic bacteria is responsible for the spread of ESBL genes.

Our study showed that theses organisms were also resistant to other commonly used antibiotics. We suggest strict antibiotic policy implementation in hospitals to reduce the spread of highly resistant bacteria, which will help to improve health condition and reduce cost to treat such complicated diseases.

REFERENCES

Ali, A.M., Rafi, S. and Qureshi, A.H., 'Frequency of extended spectrum beta lactamase producing gram negative bacilli among clinical isolates at clinical laboratories of Army Medical College, Rawalpindi' J Ayub Med Coll Abbottabad, 16(1), 2004, 35- 37.

Al-Zarouni, M., Senok, A., Rashid, F., Al-Jesmi, S.M. and Paniqrahi, D., 'Prevalence and antimicrobial susceptibility pattern of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in the United Arab Emirates,' Med Princ Pract, 17(1), 2008, 32– 36.

Astagneau, P., Fleury, L., Leroy, S., Lucet, J.C., Golliot, F., Régnier, B. and Brücker, G. 'Cost of antimicrobial treatment for nosocomial infections based on a french prevalence survey'. J Hosp Infect. 42(4), 1999, 303-312.

Babypadmini, S., and Appalaraju, B,. 'Extended spectrum-lactamases in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae* - prevalence and susceptibility pattern in a tertiary care hospital', Indian J Med Microbiol, 22(3), 2004, 172-174.

Behroozi, A., Rahbar, M and Yousefi, J.V., 'Frequency of extended-spectrum beta- lactamases (ESBLs) producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from urine in an Iranian 1000-bed tertiary care hospital', Afr J Microbiol Res, 4(9), 2010, 881-884.

Bell, J.M., Turnidge, J.D., Gales, A.C., Pfaller, M.A. and Jones, R.N, 'Prevalence of extended spectrum beta-lactamase (ESBL) -producing clinical isolates in the Asia- Pacific region and South Africa: regional results from SENTRY antimicrobial surveillance program' Diagn Microbiol Infect Dis. 43(3), 2002, 193–198.

Bradford, P.A. 'Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat'. Clin Microbiol Rev. 14(4), 2001, 933-951.

Bush, K. and Jacoby, G.A., 'Updated functional classification of β-lactamases'. Antimicrob Agents Chemother, 54(3), 2010, 969-976.

Bush, K., 'New β-lactamases in gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy', Clin Infect Dis,32(7), 2001, 1085-1089.

Frank. T., Arlet, G., Gautier, V., Talarmin, A. and Bercion, R. 'Extended-spectrum β- Lactamase- producing *Enterobacteriaceae*, Central African Republic. Emerg Infect Dis' 12(5), 2006, 863-865.

Goussard, S., and Courvalin, P., 'Updated sequence information for TEM beta-lactamase genes', Antimicrob Agents Chemother, 43(2), 1999, 367-370.

Hanberger, H., Garcia-Rodriguez, J.A., Gobernado, M., Goossens, H., Nilsson, L.E. and Struelens, M.J. 'Antibiotic susceptibility among aerobic gram-negative bacilli in intensive care units in 5 European countries. French and Portuguese ICU Study Groups', JAMA. 281(1), 1999, 67–71.

Jain. A., Roy. I., Gupta, M.K., Kumar, M and Agarwal, S. K. 'Prevalence of extended- spectrum β- lactamase-producing Gram-negative bacteria in septicaemic neonates in a tertiary care hospital. J Med Microbiol, 52, 2003, 421-425.

Jamal, W., Rotimi, V.O., Khodakhast, F., Saleem,R., Pazhoor, A. and Al-Hashim, G., 'Prevalence of extended-spectrum beta-lactamases in *Enterobacteriaceae*, *Pseudomonas* and *Stenotrophomonas* as determined by the VITEK 2 and E test systems in a Kuwait teaching hospital', Med Princ Pract. 14(5), 2005, 325–331.

Jones, R.N. and Pfaller, M.A. 'Bacterial resistance: a worldwide problem'. Diagn Microbiol Infect Dis, 31(2), 1998, 379–388.

Khan,E., Ejaz, M., Zafar, A., Jabeen, K., Shakoor,. S., Inayat, R. and Hasan, R. 'Increased isolation of ESBL producing *Klebsiella pneumoniae* with emergence of carbapenem resistant isolates in Pakistan: report from a tertiary care hospital' J Pak Med Assoc, 60(3), 2010; 186,190.

Khan, J.A, Iqbal, Z., Rahman, S.U., Farzana, K. and Khan, A., 'Prevalence and resistance pattern of *Pseudomonas aeruginosa* against various antibiotics. Pak J Pharm Sci. 21(3), 2008, 311- 315.

Knothe, H., Shah, P., Krcmery, V., Antal, M., and Mitsuhashi, S., 'Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of *Klebsiella pneumoniae* and *Serratia marcescens'* Infection, 11(6), 1983, 315-317.

Lautenbach, E., Patel, J.B., Bilker,W.B., Edelstein, P.H. and Fishman, N.O. 'Extended- spectrum β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes'. Clin Infect Dis. 32(8), 2001, 1162–1171.

Lewis, M.T., Yamaguchi, K., Biedenbach, D.J. and Jones, R.N., 'In vitro evaluation of cefepime and other broad-spectrum beta-lactams in 22 medical centers in Japan: a phase II trial comparing two annual organism samples. The Japan Antimicrobial Resistance Study Group'. Diagn Microbiol Infect Dis, 35(4), 1999, 307–315.

Luzzaro, F., Mezzatesta, M., Mugnaioli, C., Perilli, M., Stefani, S., Amicosante, G., Rossolini,G.M. and Toniolo, A., 'Trends in production of extended-spectrum beta-lactamases among *Enterobacteria* of medical interest: report of the second Italian nationwide survey' J Clin Microbiol. 44(5). 2006, 1659–1664.

Moland, E.S, Sanders, C.C. and Thomson, K.S., 'Can results obtained with commercially available MicroScan Microdilution panels serve as an indicator of β-lactamase producing *Escherichia coli* and *Klebsiella* isolates with hidden resistance to expandedspectrum cephalosporins and aztreonam? J Clin Microbiol. 36(9),1998, 2575-2579.

Otman, J., Cavassin, E.D., Perugini, M.E. and Vidotto, M.C., 'An outbreak of extended- spectrum beta-lactamase-producing *Klebsiella* species in a neonatal intensive care unit in Brazil' Infect Control Hosp Epidemiol, 23(1), 2002, 8–9.

Paterson DL & Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev. 18(4), 2005, 657–686.

Philippon, A., Labia, R., and Jacoby, G., 'Extended-spectrum beta-lactamases. Antimicrob Agents Chemother', 33(8), 1989, 1131-1136.

Qayyum, S., Sattar, A. and Waqas, B. 'Hospital acquired infections; knowledge about it and its prevention' Professional Med J, 17(2), 2010, 168-173.

Sader, H.S., Gales, A.C., Granacher, T.D., Pfaller, M.A., and Jones, R.N., 'Prevalence of antimicrobial resistance among respiratory tract isolates in Latin America: results from SENTRY antimicrobial surveillance program', Braz J Infect Dis. 4(5), 2000, 245–254.

Sanders, C.C and Sanders, W.E. 'Beta-lactamase in gram-negative bacteria: global trends and clinical impact'. Clin Infect Dis, 15(5), 1992, 824-839.

Schwaber, M.J., Raney, P.M., Rasheed, J.K., Biddle, J.W., Williams, P., McGowan, J.R. J.E and Tenover, F.C. 'Utility of NCCLS guidelines for identifying extended-spectrum β- lactamases in non- *Escherichia coli* and non-*Klebsiella spp.* of *Enterobacteriaceae'*. J of Clin Microbiol. 42(1), 2004, 294-298.

Shah,A.A., Hasan, F., Ahmed, S. and Hameed, A., 'Prevalence of extended spectrum B- lactamases in nosocomial and outpatients (ambulatory) '. Pak J Med Sci, 19(3), 2004,187–191.

Shaikh, J.M., Devrajan, R.B. and Shah A.Z.S. 'Frequency, pattern and etiology of nosocomial infection in intensive care unit: an experience at a tertiary care hospital' J Ayub Med Coll Abbottabad, 20(4), 2008, 1-5.

Shukla, I., Tiwari, R., and Agrawal, M., 'Prevalence of extended spectrum-lactamase producing *Klebsiella pneumoniae* in a tertiary care hospital'. Indian J Med Microbiol, 22, 2004, 87- 91.

Subha, A. and Ananthan, S., 'Extended spectrum beta lactamase (ESBL) mediated resistance to third generation cephalosporins among *Klebsiella pneumoniae* in Chennai'. Indian J Med Microbial, 20, 2002, 92-95.

Ullah, F., Malik, S.A., and Ahmed, J., 'Antibiotic susceptibility pattern and ESBL prevallence in nosocomial *Escherichia coli* from urinary tract infections in Pakistan', Afr J Biotechnol. 8(16), 2009, 3921-3926.