Innovations in Pharmaceuticals and Pharmacotherapy

www.innpharmacotherapy.com

eISSN: 2321–323X

Research Article

Isolated multidrug-resistant ESBL enterobacteriaceae strains in wounds are susceptible to amikacin

Ravichandran B, Thyagarajan Ravinder, Radhika Katrakadda, K. V. Leela

Govt. Kilpauk Medical College, Chennai, Tamilnadu, Dr. MGR Medical University, Chennai, Tamilnadu, India

Abstract

Enterobacteriaceae is a family of facultative aerobic bacteria thriving well in wounds that are prone to hospital borne infections infections, in specific post-operative wounds. Recent observations reflect the increased incidence and prevalence of ESBL (Amber's class A Penicillinases) that are resistant to 3rd generation cephalosporins (extended spectrum cephalosporins) and aztreonam. The major ESBL producing microbes isolated in these wound infections *Escherichia coli* and *Klebsiella pneumoniae*. As random use of antimicrobials effective against these strains pose a threat to increase in virulence and resistance in these strains, a systematic survey was done to identify the rate of incidence ESBLs in diverse infection types and trending susceptibility pattern to antibiotics is a routine need. From the profiled microorganisms in the 289 patients sampled in the hospital using routine microbiological techniques, Enterobacteriaceae isolates were screened for ESBL production and antibiotic susceptibility using disk diffusion tests. Results indicated that ESBL producers are more prevalent in burns, surgical site infections and abscess, but not in traumatic wounds; *Klebsiella pneumoniae* (42.5 %) were more prevalent than *Escherichia coli* (40%) and were more sensitive to amikacin. This kind of study ensures judicious usage of antibiotics to prevent development of new resistance mechanisms by the ESBL producers.

Keywords: ESBL producers, Klebsiella pneumoniae, Escherichia coli, 3rd generation cephalosporins, wound infections

***Corresponding author: Ravichandran,** Govt. Kilpauk Medical College, Chennai, Tamilnadu, Dr. MGR Medical University, Chennai, Tamilnadu, India. E- mail: drrashy1974@yahoo.co.in

1. Introduction

Skin is the largest organ in the human body and plays a crucial role in the sustenance of life not only through the regulation of water and electrolyte balance or thermoregulation, but also by acting as a natural barrier to the external noxious agents including microorganisms. However, when the epithelial integrity of skin is disrupted by trauma or through surgical procedures a wound result [1], which provides an optimal environment for microbial colonization and proliferation [2]. This has been estimated that the infected wound stay takes nearly 6-10 days more than an uninfected wound to heal [3].

The wound infection depends on a complex interaction between the host factors like immunity, nutritional status and age, and the wound related factors like magnitude of trauma, dead space, devitalization and presence of hematoma, and microbial invasiveness, toxins secreted and resistance to antibiotics [4]. They are classified into two major categories: skin and soft tissue

infections, and can overlap in the process of disease progression [5]. Endogenous wounds and abscess may be associated with appendicitis, cholecystitis, cellulitis, dental infection, septic arthritis, osteomyelitis, empyema and sinusitis. Most of these processes are nosocomial contracted after invasive procedures, surgical manipulation and placement of prosthesis [6]. The potential wound pathogens in these cases and in deep wounds are Gram-negative bacilli belonging to the Enterobacteriaceae family like *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus* sp., *Enterobacter* sp.; and anaerobes like bacteroides and clostridial species [7].

With the rapid spread of resistant microbes, the effectiveness on the use of antimicrobials is becoming a worldwide problem [8]. The condition is serious in developing countries owing to irrational prescription of antimicrobial agents [9]. Thus, the battle between bacteria and their susceptibility to drugs is a challenge to the public, researchers, clinicians and drug companies to suppress the increasing demand to find novel and effective drugs. As a result, measures to control the problem will include development of new antimicrobial, better infection control program and more appropriate use of existing antimicrobial agents [10, 11, 12]. Many researchers made different recommendations on the susceptibility of microorganisms to drugs [13].

In recent years the increased incidence and prevalence of ESBL (Amber's class A Penicillinases) that hydrolyze and cause resistance to oxyamino cephalosporins (extended spectrum cephalosporins) and aztreonam [14, 15] have been found to be in significant percentage of *Escherichia coli* and *Klebsiella pneumoniae*. These strains were also identified in *Pseudomonas aeruginosa* and other Enterobacteriaceae strains like *Enterobacter* sp., *Citrobacter* sp., *Proteus* sp., *Morganello morganii*, *Serratia marsescens* and *Shigella dysenteriae* [16]. The production of these enzymes is either chromosomally mediated or plasmid mediated with pointed amino acid substitution of the classical plasmid mediated beta lactamases like TEM-1, TEM-2 and SHV-1thereby increasing the spectrum of activity from earlier generation beta lactams to 3rd generation cephalosporins and monobactams. However, they retain their stability against cephamycins and carbapenems and are inhibited by beta lactamase inhibitors (clavulanic acid, sulbactam and tazo bactam). Today over 575 different ESBLs have been described [17]. As these are plasmid mediated, the enzymes are transferred to other bacterial species resulting in afflicting of the infection control, clinical and therapeutic procedures.

Thus, this study aims to find out common bacterial isolates and their antibiotic resistance pattern for the prevalent ESBL producers. The microflora of the wound infections from the sample populations are identified, profiled and grouped as ESBL and non-ESBLA producers for the family Enterobacteriaceae. These ESBL producers are further subjected for antibiotic susceptibility testing to choose the effective antibiotic regime.

2. Materials and methods

2.1 Collection of specimens

2.1.1. **Pus:** The area over the abscess was wiped with sterile saline or 70% alcohol using a sterile syringe and needle to aspirate the pus into a sterile test tube.

2.1.2. **Swab:** The wound was wiped with sterile saline and two swabs, one for smear and another for culture, were rolled along the leading edge of the wound and placed in a sterile test tube.

2.1.3. **Tissue bits:** For chronic wounds, the wound area was wiped with sterile saline and tissue bits were collected using sterile punch biopsy forceps into a sterile test tube filled with sterile saline to keep the specimen moist.

2.2 Specimen processing

As soon as the specimen reaches the laboratory, smears were prepared using the swab or purulent material on a clean glass slide. Tissue specimens were ground or minced using sterile scissors and forceps before processing. Gram stain of the smears were prepared and observed under a microscope.

The specimens were inoculated onto blood agar and Mac Conkey agar plates and incubated aerobically at 37° C for 18-24 hours. The micro -

organisms were identified based on colony morphology, Gram stain, motility and biochemical reactions.

2.3 Antimicrobial susceptibility testing

Routine disk susceptibility testing of the Eneterobacteriaceae isolates was performed by Kirby-Bauer method on Mueller-Hinton agar medium obtained from Himedia. 25 ml of the prepared medium was poured onto a Petri dish of 90 mm diameter to obtain a thickness of 4mm.

2.3.1. Preparation of 0.5 McFarland's turbidity standard for inoculum preparation

0.05 ml of 1% barium chloride solution was added to 9.95 ml of 1% sulphuric acid in a test tube with constant stirring to prepare a uniform suspension. The barium sulphate suspension was transferred into a 4 - 6 ml screw - capped tube similar to those used for growing and diluting the bacterial inoculum. The tube was tightly sealed and stored in refrigerator and was shook vigorously until all the deposits were raised into uniform suspension.

2.3.2. Preparation of inoculum and inoculation [18]

Morphologically similar colonies on an agar medium were touched with a sterile wire loop and the inoculum was transferred to a test tube containing 1.5 ml of nutrient broth. The tube was incubated at 35° C until the density of the inoculum is equivalent to 0.5 McFarland's standard that corresponds to 150 million organisms per ml. Within 15 minutes of preparation of the suspension, a sterile cotton wool swab was dipped into the suspension and was inoculated by even streaking on the dried surface of MHA plate. After 3 to 5 minutes, the antibiotic disks were placed and pressed intact on the inoculated agar medium.

2.3.3 Antibiotic disks

For Enterococci, the antimicrobial susceptibility testing included Penicillin 10U, Erythromycin 15 µg and Amikacin 30 µg disks. For Gram-negative bacilli, Ampicillin 10µg, Cotrimoxazole 25 µg, Ciprofloxacin 5 µg, Cefotaxime 30 µg, Ceftazidime 30 µg, Gentamicin 10 µg and Amikacin 30 µg disks and for ESBL producers Imipenem disk were used.
Antibiotic disks were applied using forceps and pressed gently to ensure even contact with the medium. The plates were inverted and incubated at 35°C to 37°C for 16 to 18 hours [19].

2.3.4 Reading zones of inhibition

The diameters of the zones of complete inhibition were measured, including the diameter of the disc in millimeter using an ordinary ruler held on the back of the inverted Petri plate. The Petri plate was held a few inches above a black non-reflecting background and illuminated with reflected light. The size of the zones of inhibition were interpreted by referring to the NCCLS (Table -2 Volume 20: No. 1, 2000) for Zone Diameter Interpretive Standards and reported as susceptible, intermediate or resistant to the antimicrobial agents tested.

2.4 ESBL detection

Enterobacteriaceae isolates with zone of inhibition diameter < 27 mm for cefotaxime and < 22 mm for ceftazidime were further tested for ESBL production adopting the following methods:

2.4.1 Double disk diffusion synergy test

In the DDST, synergy was determined between a disk of Augmentin (20 µg Amoxycillin and 10 µg Clavulanic acid) and a 30 µg disk of 3rd generation Cephalosporin (Ceftazidime) placed at a distance of 15 mm apart (center to center) on a lawn culture of the resistant isolate on MHA plate. The presence of a clear extension of the edge of zone of inhibition for the 3rd generation cephalosporin toward the disk containing Clavulanate was interpreted as synergistic indicating ESBL production. This extension occurs due to the Clavulanate in the Augmentin disk that inactivates the ESBL produced by the test organism.

2.4.2 Agar dilution method

Mueller-Hinton agar was prepared in flasks, autoclaved and cooled in a 50°C water bath. The serial dilution of 3rd generation Cephalosporins (Ceftazidime and Cefotaxime) was prepared in sterile distilled water to give a final concentration

ranging from 2 µg to 2048 µg/ml. After adding the drugs to medium at 50 °C, it was mixed well and poured into sterile Petri dishes. A control plate containing the test medium without the antibiotic was prepared for each series of test. Plates of various concentrations was divided into nine quadrants and 0.003 ml inoculum that was equivalent to the standard 0.5 McFarland solution was transferred to the appropriate quadrant and incubated at 37°C for 16 - 20 hours. Nine to twelve organisms were thus tested using a single plate. MIC was noted as the lowest concentration at which no visible growth occurred.

2.4.3 Phenotypic confirmatory test

Antibiotic sensitivity testing was done on MHA against 0.5 McFarland standard of the microorganism. The drugs used were Cefotaxime and Ceftazidime with each 30µg alone, and in combination with Clavulanic acid 10 µg. Microorganisms with zones of inhibition that increased greater than 5 mm for third generation cephalosporin and Clavulanic acid were confirmed as ESBLs. Control strains of non-ESBL producing microorganism (*Escherichia coli* ATCC 25922) and ESBL producing microorganism (*Klebsiella pneumonia* ATCC 700603) were used.

2.5 Statistical analyses

A statistical analyses was carried out using statistical package for social sciences (SPSS) and Epi-info software using a statistician. The proportional data of the cross sectional study was tested using Pearson's Chi-square analysis test and Binomial proportion test.

3. Results

3.1 Distribution of microorganisms:

Specimens obtained from patients with wound infection attending surgical, orthopaedic, burns, OG, IMCU and plastic surgery departments as OP and IP were observed between March 2009 and February 2010 to update the bacteriological profile of wound infections, antimicrobial susceptibility pattern of the isolated microorganisms and for prevalence of ESBL producing Enterobacteriaceae and their antibiotic susceptibility pattern. This study included patients of both sexes and upto 80 years of age and specimens like pus, wound swab and tissue samples were collected. Total number of cases observed was 289, of which 143 were male (49.48%) and 146 were female (50.51%) with the maximum cases falling in the age group of 21-30. In all age groups, except in the groups of 11-20 and 21-30, the sex distribution was predominantly male.

Out of 289 samples collected, 164 showed culture positive. The isolated organisms were *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococci* sp., coagulase-negative *Staphylococcus* sp. and *Acinetobacter* sp as in Table 2. Of these, *Klebsiella pneumoniae*, *Escherichia coli* and *Enterococci* sp. are the members belonging to the Enterobacteriaceae family. In all kinds of wound infections like burn wounds, surgical site infections, cutaneous abscesses and traumatic wounds, *Klebsiella pneumoniae* was predominant – 13 (11.71 %), 7 (26.92 %), 5 (22.72 %) and 1 (20 %), respectively followed by *Escherichia coli* in the following proportions – 1 (.09 %), 2 (7.6 %) and 2 (9.09 %; and none were isolated in traumatic wounds).

3.2 Antibiotic sensitivity pattern for the Enterobacteriaceae family:

Out of 5 isolates of the *Enterococci* sp., 4 (80%) were sensitive to ciprofloxacin, 3 (60%) were sensitive to amikacin, 2 (40%) were sensitive to gentamicin, erythromycin, cefotaxime, cephalexin, levofloxacin and all 5 (100%) were sensitive to vancomycin. Of the 40 isolates of *Klebsiella pneumoniae*, 36 (90%) were sensitive to amikacin, 34 (85%) were sensitive to ciprofloxacin, 10 (25%) were sensitive to cefotaxime, 5 (12.5%) were sensitive to cephalexin, 20 (50%) were sensitive to Piperacillin/tazobactum, 24 (60%) were sensitive to imipenem, and 18 (45%) were sensitive to gentamicin (Table 2).

Of the 5 isolates of *Escherichia coli*, 4 (80%) were sensitive to amikacin, imipenem, 3 (60%) were sensitive to ciprofloxacin, 2 (40%) were sensitive to cephalexin and 1 (20%) were sensitive to cefotaxime, piperacillin/tazobactum and gentamicin.

3.3 Screening for ESBL

Of the 45 Enterobacteriaceae isolates, 19 (42.22%) isolates were found to be ESBL producers using the screening method of resistance to 3rd generation cephalosporin and were further confirmed as ESBL producers using the confirmatory tests DDST, MIC and PCT (Table 3 and Table 4). Of these 19 isolates identified as ESBL producers, 17 isolates were *Klebsiella pneumoniae* (42.50 %) and 2 isolates were *Escherichia coli* (40 %) (Table 5). As in Table 6, the ESBL isolates were prevalent in burns, surgical site infections and cutaneous abscesses; and none was identified in traumatic wounds.

3.4 Antibiogram of ESBL producers

The ESBL producers were resistant to antibiotics like cephalexin (95 %), cefotaxime (89.5 %), piperacillin /tazobactum (79 %), gentamicin (58 %), ciprofloxacin (58 %), amikacin (10.5 %), and imipenem (58 %) (Table 7). MIC pattern for the isolates was between 32µg/ml to 2048µg/ml of agar for cefotaxime and ceftazidime was found to be reduced from 0.125 to 128 µg/ml of agar in the presence of 2 µg of clavulanic acid/ml of agar as in Table 8 & 9.

4. Discussion

The control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics and to greater incidence of infections caused by ESBL producing strains. The clinical microbiological laboratory has the task of screening and confirming isolates for ESBL production and assessing their antibiotic susceptibility that plays an important role in the treatment of wound infections.

Extended spectrum beta-lactam antimicrobial drugs are commonly included in empirical antibiotic regimen for treatment against Gram-negative sepsis, but the emergence of ESBL producing bacteria poses a serious threat to the continued use of this family of antibiotics [20.] Therefore, infections caused by ESBL isolates need to be addressed with a general consensus in order to overcome the challenge of infection management against development of antibiotic resistance worldwide.

There have been sporadic reports of ESBLs from major hospitals in India and some of them recorded the incidence to be as high as 60 - 68% [21], an unusually high incidence of ESBLs raising a concern to the regulators of the hospital antibiotic use policy. Over reliance on third generation cephalosporins to treat Gram- negative infections is one of the prime factors responsible for increased resistance to this class of antibiotics.

The 164 isolates identified (56.74%) in the present study had 45 Enterobacteriaceae isolates, of which 19 (42.22%) isolates were ESBL producers. Similar studies by C Rodrigues *et al* [22], the prevalence of ESBL was 53%; while the occurrence of ESBL producing *Klebsiella pneumoniae* accounts for 42.5 % and *Escherichia coli* was 40%. This correlates with the study of Ashwin *et al* [26], 43.75% and 58.06% respectively. ESBLs amongst *Klebsiella pneumoniae* isolates of this study correlates with Leblebicioglu [27] (50%) and Ozgunes (47 %) [28]. In contrast, a study by Shukla *et al* [29] showed only 36.1% ESBL producing *Klebsiella pneumoniae* isolates. A similar case of lower incidence of ESBL producing *Klebsiella pneumoniae* isolates (14 %) were observed by MS Kumar *et al* [27], in which he also observed a higher incidence of ESBL producing *Escherichia coli* (63.7 %). In another study by Rezwana Haque *et al* [27], the percentage of ESBL producing bacteria was 46.67 % and the highest rate was found in *Klebsiella pneumoniae* (57.89%) followed by *Escherichia coli* (47.83%), which correlates with our study ESBL was 42.22% and Klebsiella pneumoniae (42.50%) and Escherichia coli (40%).

85 % of *Klebsiella pneumoniae* exhibited MIC of 256 µg/ml to cefotaxime and 95 % of *Escherichia coli* exhibited MIC of 256 µg/ml to cefotaxime in the current study. In this study by Shukla *et al* [26], the prevalence of ESBL producing *Klebsiella pneumoniae* was 30.18 % and the MIC of 3rd generation cephalosporins test antibiotics against ESBL producers ranged between 2 to 128 µg/ml. In our study, 89.5 % of ESBL positive bacterial strains were sensitive only to amikacin, while they showed significant resistance to all other antibiotics tested implying that the isolated ESBL producing microorganisms are multidrug resistant. The prevalence of these multidrug resistant ESBL strains was also reported to be on the rise [29].

The resistance pattern of ESBL producers in our study were 79 % to piperacillin/tazobactum

and 58 % to gentamicin , ciprofloxacin, imipenem but 89.5 % sensitive to amikacin, which correlates with study by Baby Padmini *et al* [30] where the sensitivity of ESBL producers to amikacin was 82.6 %. In the study by Dechen C Tsering *et al* [31] the prevalence of ESBL was 34 % and 51.9 % were resistant to piperacillin/ tazobactum and ciprofloxacin, and 54.3% were resistant to gentamicin. The resistance pattern of ESBL producers in a study by Rezwana Haque *et al* [28] was 100% to ampicillin, 81.82% to ciprofloxacin, 45.45% to gentamicin but 100% sensitive to imipenem. Jyoti Sonawane *et al* [32] found the ESBL isolates were frequently resistant to other antibiotics but showed nearly to 100% sensitivity to piperacillin/tazobactum and imipenem.

Minimum inhibitory concentrations (MIC) of Cefotaxime and Ceftazidime and of Cefotaxime and Ceftazidime with 2 µg/ml of Clavulanic acid by agar dilution method for ESBL producers (N=19) was between 32-2048 mg/ml of agar. MIC of cefotaxime for ESBL producing isolate was between 0.125 -128 mg/ml of agar in the presence of clavulanic acid at a concentration of 2 mg/ml of agar showing 8 fold reductions in MIC. MIC of ceftazidime for ESBL producing isolate was between 0.125 -128 mg/ml of agar in the presence of clavulanic acid at a concentration of 2 mg/ml of agar showing 8 fold reductions in MIC.

Monitoring and judicious usage of extended spectrum cephalosporins, periodic surveillance of antibiotic resistance patterns and efforts to decrease empirical antibiotic therapy would go a long way in addressing some of the problems associated with ESBLs.

Antibiotics	*Klebsiella pneumoniae* N=40 (percentage)
Amikacin	36 (90)
Ciprofloxacin	34 (85)
Cephatoxime	10 (25)
Cephalexin	5 (12.5)
Piperacillin/tazobactum	20 (50)
Imipenem	24 (60)
Gentamicin	18 (45)

Table 1: Distribution of microorganisms

Organisms	No of isolates	Percentage
Staphylococcus aureus	89	54.26
Klebsiella pneumoniae	40	24.39
Pseudomonas aeruginosa	22	13.41
Escherichia coli	5	3.04
Enterococci sp.	5	3.04
Coagulase-negative *Staphylococcus* sp.	2	1.21
Acinetobacter sp.	1	0.6

Table 2: Sensitivity pattern of *Klebsiella pneumonia*

Organisms	Total	ESBL	Percentage
Klebsiella pneumoniae	40	17	42.50
Escherichia coli	5	2	40
Total	45	19	41.25

Table 3: No of enterobacteriaceae resistant to 3rd generation cephalosporins

Number of isolates	Resistant to 3rd generation cephalosporins
45	19

Table 4: ESBL detection by various methods

Methods	DDST	MIC	PCT
Positive isolates n=19	19 (100%)	19 (100%)	19 (100%)

Table 5: Distribution of ESBL producing enterobacteriaceae

Organisms	Total	ESBL	Percentage
Klebsiella pneumoniae	40	17	42.50
Escherichia coli	5	2	40
Total	45	19	41.25

Table 6: Distribution of ESBL producing enterobacteriaceae from various wounds

Organism	Burns		SSI		Traumatic wound		Abscess		Total	
	Total	ESBL	Total	ESBL	Total	ESBL	Total	ESBL	Total	ESBL
Klebsiella pneumoniae	29	12	7	3	2	0	2	2	40	17
Escherichia coli	1	1	2	1	-	-	2	-	5	2
Total	30	13	9	4	2	-	4	2	45	19

Table 7: Resistance pattern of ESBL producers to other antibiotics

Antibiotics	ESBL Producers (N=19) %
Amikacin	10.5
Ciprofloxacin	58
Cefotaxime	89.5
Cephalexin	95
Piperacillin/tazobactum	79
Imipenem	58
Gentamicin	58

Table 8: MIC of enterobacteriaceae to cefotaxime and cefotaxime with clavulanic acid

Cefotaxime µG/ML	NO of Isolates Inhibited	Cefotaxime & Clavulanic acid (2µG/ML)	No of isolates inhibited
1	-	0.125	1
2	-	0.25	3
4	-	0.5	5
8	-	1	4
16	-	2	7
32	3	4	4
64	3	8	1
128	5	16	1
256	4	32	-
512	6	64	-
1024	8	128	2
2048	9	256	-

Table 9: MIC of enterobacteriaceae to ceftazidime and ceftazidime with clavulanic acid

Ceftazidime µG/ML	No of isolates inhibited	Ceftazidime & clavulanic acid (2µG/ML)	No of isolates inhibited
1	-	0.125	2
2	-	0.25	3
4	-	0.5	5
8	-	1	8
16	-	2	7
32	5	4	3
64	6	8	1
128	9	16	-
256	9	32	-
512	8	64	-
1024	5	128	-
2048	4	256	-

Reference

1. Enoch, s. and price, p.2004. Cellular and biochemical differences in the Pathophysiology of healing between acute wounds, chronic wounds, and wounds in the aged.

2.Bowler PG, Duerden BI, Armstrong DG.2001. Wound microbiology and associated approaches to wound management. Clin. Microbiology review 14;244-269

3. Plowman, r.2005. The socioeconomic burden of hospital acquired infection. Euro.surveill; 5(4);49-50

4. Kernodle ds. Kaiser ab. Postoperative infections and antimicrobial prophylaxis. In; Mandell gl. Bennett je, Dolin r eds. Principle and practice of infectious diseases. 4th edition newyork, Churchill livingstone 1995; 2742-56

5. Hackner sm 1994. Common infection of the skin; characteristics, causes and cures.post graduate medicine. 96;43-529.

6. Konemans color atlas and Text book of diagnostic microbiology, 6th edirion 2006. Lippincott Williams and wilkins..

7. Cooper R, Kingsley A, White R. *Wound Infection and Microbiology. :* Medical Communications (UK) Ltd for Johnson & Johnson Medical, 2003.

8. Mulu a, Moges f , Tessema b, Kassu a. Pattern and multiple drug resistance of bacterial pathogens isolated from wound infection at university of Gondar teaching hospital, north west Ethiopia. Ethiop med j. 2006; 44 (2); 125-131

9. Shears p. Antimicrobial resistance in tropics. Tropical doctor. 2003; 30(2); 114- 116

10. World health organization. Resistance to antimicrobial agent. WHO bulletin.1996;74(3);335-336

11. Turnidge j. What can be done about resistance to microbes? Br Med J.1998;317;645-647

12. Hart ca, Kairuki s. Antimicrobial resistance in developing countries.Br Med J 1998;317; 640-650

13. Rind r, Khan ts. Antibiogram sensitivity of bacterial organisms identified from surgical and non surgical wounds of animal. Pak J Bio.Sci.2000; 3(10); 1719-1723

14. Bradford . ESBL s in the 21st century; characterization, epidemiology and detection of this important resistance threat. Clinic Micro Rev 2001;14(4) 933-951

15. Rice , Bonomo, A betalactamases which one are clinically important? Drug resistance update.2000;3;178-189

16.Thomson. Controversies about extended spectrum and amp_c betalactamases. Emerging Infectious Dis 2001;7(2) 333-336

17. www.lahey.org maintaining the list of Extended spectrum betalactamases.

18. Konemans color atlas and Text book of diagnostic microbiology, 6th edirion 2006. Lippincott Williams and wilkins..pp945.

19. Mackie and Mc Cartney Practical medical microbiology 14th edition 2006 churchill livingstone pp166-169

20. Livermore DM. ß-lactamases in laboratory and clinical resistance. Clin Microbiol Rev 1995;8:557–584

21. Mathur p , Tatman A, Das B, Dhawan B.Prevalence of ESBL producing bacteria in a tertiary care hospital, Indian journal of medical research 2002 115 153-157

22. C Rodrigues P Joshi, SH Jani, M Alphonse, R Radhakrishnan, A Mehta. Detection of lactamases in nosocomial gram negative clinical isolates IJMM 2004 22 4 247-250

126, 127 and 128 missing…

26. Shukla I,Tiwari R, Agarwal M. Prevalence of ESBL producing klebseilla pneumonia in tertiary care hospital, IJMM 2004, 22, 287-291.

27. MS Kumar V lakshmi, R Rajagopalan. Occuernce of ESBLs among Enterobacteriaceae isolated at teriary care institute, IJMM, 2006, 24, 3, 208-211.

28. Rezwana Haque M.A Salam .Detection of ESBL producing nosocomial gram negative bacteria from a tertiary care hospital in Bangladesh Pak J Med Sci 2010 vol26 no.4 887-891.

29. Hyle EP, Lipworth AD, Zaoutis TE, Nachamkin I, FishmanNO, Bilker WB, et al. Risk factors for increasing multidrugresistance among extended spectrum betalactamase producing Escherichia coli and Klebsiella species. Clin Infect Dis 2005;40:1317-1324.

30.S Baby padmini , B Appala Raju, KR M ani. Detection of Enterobacteriaceae producing CTX-M ESBLs from a tertiary care hospital in South India, IJMM 2008 26,2,163-166

31. Dechen C Tsering , Shyamasree Das, Luna Adhiakari, Ranabil Pal, Takhellambam S K Singh. Extended spectrum beta-lactamase detection in gram-negative bacilli of nosocomial origin Journal of Global Infectious Disease. 2009 1 2 87-92

32. Jyoti sonawane, Narayan Kamath, Rita Swaminathan, kausal Dhosani. Bacterial profile of SSI and their antibiograms in a tertiary care hospital in Navi Mumbai., Bombay hospital journal 2010, 52, 3.