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Prevalence of ESBL producing Gram Negative Bacilli in Post Operative wound Infections

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A B S T R A C T

To study the aerobic Gram Negative bacilli in Postoperative Wound Infections with special reference to Extended Spectrum β lactamases (ESBL). The study was conducted on 173 patients who underwent surgery in General Surgery department. Pus samples were collected with two sterile swabs and processed in Microbiology department by Standard methods. The overall surgical wound infection rate was 5.97%. of the 173 pus samples, 127(73.41%) showed growth, among these Gram Negative aerobic bacilli isolated was 79 (62.2%). Pseudomonas aeruginosa (31.6%) was the most predominant organism followed by *Escherichia coli* (22.7%) which, in turn followed by *Klebsiella pneumoniae* (18.9%). Out of the 79 isolates which were screened for ESBL production, 25(31.6%) isolates were found to be ESBL positive by CLSI disc diffusion and 23(29.1%) were positive by screening with HiCrome ESBL agar. On performing confirmatory tests on the 25 isolates which were ESBL positive by screening tests, 23 (92%) were found to be ESBL producers on CLSI phenotypic confirmatory test and 22 (88%) were found to be ESBL producers on Double Disc Synergy Test. The prevalence of ESBL producing gram negative bacilli in postoperative wounds is 29.1% which were detected by screening and confirmatory tests.

Keywords: Surgical site infection (SSI), Extended spectrum beta lactamase (ESBL), Phenotypic confirmatory test (PCT), Clinical Laboratory Standards Institute (CLSI)

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1. Introduction

Antibiotics have always been considered one of the wonder discoverer of the 20th century. This is true, but the real wonder is the rise of antibiotic resistance in hospitals, communities and the environment concomitant with their use. Surgical site infections constitute about one fourth of all nosocomial infections. They are an important cause of morbidity and mortality following various surgeries and also account for additional costs. Most nosocomial surgical site infections (60-80%) occur in the incision, but some involve deep soft tissue or adjacent sites [1]. Post operative wound infections have been a problem since surgery was started as treatment modality. The advancement in medicine has resulted in the prevention and the control of this infection. β lactam antibiotics are among the most over used antimicrobial agents worldwide. The emergence of resistance to these agents in the past two decades has resulted in a major clinical crisis. Gram Negative bacteria resistant to agents such as Extended Spectrum Cephalosporins, Monobactams, Carbapenems and β-lactamase inhibitor combinations emerged through the production of a variety of β-lactamases, alterations in the Penicillin binding proteins, Outer membrane permeability and combinations of multiple mechanisms of resistance. In recent years, there has been an increase in infections caused by Extended Spectrum Beta Lactamase (ESBL) producing strains of gram negative bacilli which hydrolyze and confer resistance to the Penicillins, first, second and third generation Cephalosporins and Azetreonam (but not the Cephamycins or Carbapenems and are inhibited by β-lactamase inhibitors such as clavulanic acid. They developed from point mutation of genes which code production of primordial TEM-1, TEM-2 or SHV-1 β lactamases with replacement of the configuration of amino acids at an active site for the enzymes [2]. Infections with ESBL producing bacterial strains are encountered singly or in outbreaks, especially in critical care hospitals, resulting in increasing costs of treatment and prolonged hospital stay [3]. Treatment of the infections caused by ESBL producers is complicated not only due to resistance to Extended Spectrum Cephalosporins, but also because many ESBL genes are present on large plasmid which contain genes encoding resistance to many other antibiotics including Aminoglycosides, Chloramphenicol, Sulphonamides and Tetracyclines [4]. This study was undertaken to determine the prevalence of ESBL producing gram negative bacilli in post operative wound infections and also importance of detection of these enzymes and the epidemiology.

2. Materials and Methods

This is prospective study carried out for 2 years in the department of Microbiology. Patients who underwent Clean and Clean contaminated electively and Contaminated and Dirty surgeries in an emergency were included. [(5),(6)]
Surgical wound was inspected at the time of first dressing and weekly thereafter for 30 days. Wound infection was diagnosed if nay one of the following criteria were fulfilled. Serous or pus discharge from the wound with signs of inflammation and wound deliberately opened by the surgeon due to localized pus collection. Relevant clinical history of the patient was taken. Swabs obtained from infected wounds were processed aerobically by standard methods.(7),(8) Gram Negative Bacilli isolated and were tested for ESBL production. (9) Data were evaluated by chi square ($\chi 2$) statistical test. $P \leq 0.05$ was considered be significant.

3. Results and Discussion

The overall frequency of the Surgical Site Infections (SSI) was 5.97%.

Table 1: Rate of Culture positives among clinically diagnosed SSI patients

Wounds Studied	**2124**	-
Clinically Suspected cases of SSI	**173**	8.14%
No. of Definite SSI (culture Positive)	**127**	**5.97%**

Table 2: Distribution of SSI in different wound classes

S.No	**Class of Wound**	**Clinically Diagnosed cases**
1	I(Clean Wound)	74
2	II (Clean contaminated wound)	48
3	III (Contaminated)	40
4	IV(Dirty Wound)	11
Total		**173**

Of the 173 clinically diagnosed cases of SSI, 74 (42.7%) cases belonged to class I wound, 48 (27.7%) to class II, 40 (23.1%) to class III and 11 (6.35%) cases belonged to class IV wounds.

Table 3: Age and Sex distribution of patients in relation to culture positivity

S.No.	Age Group in Years	Male		Female		Total		
		Clinically Diagnosed	Culture positive	Clinically Diagnosed	Culture positive	Clinically Diagnosed	Culture positive	%
1.	0-10	5	2	3	1	8	3	37.5
2.	11-20	14	6	4	2	18	8	44.4
3.	21-30	16	16	8	4	24	20	83.3
4.	31-40	31	27	15	12	46	39	**84.7**
5.	41-50	29	26	13	9	42	35	83.3
6.	51-60	17	9	6	3	23	12	52.1
7.	61 and above	9	8	3	2	12	10	83.3
Total		121	**94**	52	**33**	173	**127**	

Out of 173 clinically diagnosed cases of SSI, 121 (69.9%) were males and 52 (30.05%) were females. Maximum number of SSI patients were in the age group of 31-40 years. Of the 173 clinically diagnosed cases of SSI, 127 (73.41%) samples were culture positive.

Table 4: Culture positivity in relation to class of wound

S.No	Class of wound	Clinically Diagnosed cases	Culture Positive Cases	Percentage of culture positives
1	I	74	39	**52.7%**
2	II	48	40	**83.3%**
3	III	40	37	**92.5%**
4	IV	11	11	**100%**
Total		173	**127**	

The incidence of culture positive cases amongst the clean surgical cases was 52.7%, clean contaminated cases was 83.3%, Contaminated cases was 92.5% and among the Dirty cases it was 100%.

Table 5: Types of Gram Negative bacteria isolated

S.No	Organisms	Total No	Percentage
2	*Pseudomonas aeruginosa*	25	**31.6%**
3	*Escherichia coli*	18	22.7%
4	*Klebsiella pneumoniae*	15	18.9%
5	*Proteus mirabilis*	10	12.6%
9	*Citrobacter*	5	6.3%
10	*Providencia rettgeri*	4	5%
11	*Acinetobacter*	2	2.5%
Total		**79**	100

Out of 127 Culture positive samples, Gram Negative aerobic bacilli isolated was 79 (62.2%). Among Gram Negative bacilli, Pseudomonas aeruginosa (31.6%) was the most predominant organism followed by *Escherichia coli* (22.7%) which, in turn followed by *Klebsiella pneumoniae* (18.9%).

Table 6: Antibiotic sensitivity pattern of Gram Negative organisms

Organisms	AMX		PIT		CTX		CAZ		CAC		TE		COT		CIP		AK		GEN		IPM	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
***Pseudomonas aeruginosa* Total no-25**	0	25	21	4	-	-	12	13	15	10	16	9	16	9	18	7	21	4	22	3	22	3
***Escherichia coli* Total -18**	3	15	-	-	10	8	10	8	11	7	12	6	12	6	13	5	14	4	15	3	18	0
***Klebsiella pneumoniae* Total -15**	4	11	-	-	8	7	8	7	10	5	9	6	9	6	10	5	11	4	12	3	15	0

***Proteus mirabilis* Total - 10**	1	9	-	-	4	6	4	6	5	5	6	4	4	6	4	6	8	2	7	3	10	0
Citrobacter koseri Total -5	1	4	-	-	3	2	3	2	4	1	3	2	2	3	3	2	4	1	5	0	5	0
Providencia rettgeri Total -2	0	2	-	-	0	2	0	2	1	1	1	1	1	1	1	1	2	0	2	0	2	0
Acinetobacter Total -2	0	2	1	1	-	-	0	2	1	1	2	0	2	0	1	1	2	0	2	0	2	0
Total- 64	6	58	17	5	21	21	35	29	35	29	43	21	35	29	25	39	41	23	46	18	55	9

Out of 79 Gram Negative Isolates, 25 were Pseudomonas aeruginosa among which 22(88%) were sensitive to Imipenem, Gentamicin, 21(84%) were sensitive to Piperacillin-Tazobactum, Amikacin, 18(72%) were sensitive to Ciprofloxacin, 16(64%) were sensitive to tetracycline and cotrimoxazole, 15(60%) were sensitive to Ceftazidime/Clavulanic acid, 12(48%) were sensitive to ceftazidime and all are resistant to amoxicillin. Out of 18 Escherchia coli isolates, 18(100%) were sensitive to imipenem, 15(83.3%) were sensitive to gentamicin, 14(77.7%) were sensitive to amikacin, 13(72.2%) were sensitive to ciprofloxacin, 12(48%) were sensitive to tetracycline and cotrimoxazole, 11(61.1%) were sensitive to ceftazidime/clavulanic acid, 10(55.5%) were sensitive to ceftazidime, cefotaxime, 3(12%) were sensitive to amoxicillin. Out of 15 klebsiella pneumoniae isolates, all were sensitive to Imipenem, 12 (80%) were sensitive to gentamicin, 11(73.3%) were sensitive to amikacin, 10(66.6%) were sensitive to Ciprofloxacin, Ceftazidime/Clavulanic acid, 9(60%) were sensitive to Tetracycline, cotrimoxazole, 8(53.3%) were sensitive to cefotaxime, ceftazidime, 4(26.6%) were sensitive to amoxycillin. Out of 10 Proteus mirabilis isolates, all were sensitive to imipenem, 8(80%) were sensitive to amikacin, 7(70%) were sensitive to gentamicin, 6(60%) were sensitive to tetracycline, 5(50%) were sensitive to ceftazidime/clavulanicacid, 4(40%) were sensitive to ciprofloxacin, cotrimoxazole, ceftazidime, cefotaxime, and 1(10%) were sensitive to amoxicillin.Out of 5 Citrobacter koseri isolates, all were sensitive to Imipenem, gentamicin, 4(80%) were sensitive to amikacin, ceftazidime/clavulanicacid, 3(60%) were sensitive to ciprofloxacin, tetracycline, Ceftazidime, cefotaxime, 2(40%) were sensitive to cotrimoxazole, and 1(20%) was sensitive to amoxycilin. Out of 2 Providencia rettgeri isolates, all were sensitive to Imipenem, Amikacin, Gentamicin, 1(50%) were sensitive to Tetracycline, Cotrimoxazole, ciprofloxacin, ceftazidime/clavulanic acid, and all were resistant to cefotaxime, ceftazidime, amoxicillin. Out of 2 Acinetobacter isolates, all were sensitive to Imipenem, Amikacin, Gentamicin, tetracycline, cotrimoxazole, 1 (50) were sensitive to Piperacillin/Tazobactum, Ceftazidime/clavulanic acid, and all were resistant to ceftazidime, amoxicillin.

Table 7: Screening tests for ESBL by CLSI disc diffusion and HiCrome agar

Organism	**CLSI screening by disc diffusion**	**Screening with HiCrome agar**
Pseudomonas aeruginosa	8	8
Escherichia coli	7	6
Klebsiella pneumoniae	5	4
Proteus spp	2	2
Citrobacter spp	1	1
Providencia rettgeri	2	2
Acinetobacter spp	0	0
Total	25	23

Out of the 79 isolates which were screened for ESBL production, 25(31.6%) isolates were found to be ESBL positive by CLSI disc diffusion and 23(29.1%) were positive by screening with HiCrome ESBL agar.

Table 8: Rate of detection of ESBL by phenotypic CLSI confirmatory test and DDST

Organism	**No. tested**	**PCT**		**DDST**	
		No.	%	No.	%
Pseudomonas aeruginosa	8	8	32%	8	32%

Escherichia coli	7	6	33.3%	6	33.3%
Klebsiella pneumoniae	5	4	26.6%	4	26.6%
Proteus spp	2	2	20%	2	20%
Citrobacter spp	1	1	20%	1	20%
Providencia rettgeri	2	2	50%	1	25%
Acinetobacter spp	0	0	-	0	-
Total	25	23		22	

PCT = CLSI phenotypic confirmatory test by disc diffusion
DDST = Double disc synergy test

On performing confirmatory tests on the 25 isolates which were ESBL positive by screening tests, 23 (92%) were found to be ESBL producers on CLSI phenotypic confirmatory test and 22 (88%) were found to be ESBL producers on Double Disc Synergy Test.

Discussion

Of 79 Gram Negative aerobic bacilli isolates 23(29.1%) were found to be ESBL producers, which were screened and confirmed by CLSI methods. This study, showed a prevalence rate of 29.1% of the total isolates of ESBLS, in post operative wound infection (world wide prevalence <1 to 74%). In India the prevalence rate varies from 28 to 84% whereas is the U.S it was 0 to 25% and the national average is 3% (CDCNNIS) The high percentage of ESBL producing isolates (29.1%) deducted in this study may be due to selective pressure imposed by extensive use of antimicrobials in the ICU ward and Post operative wards. [10]The prevalence rate of ESBL detected in this study was 29.1%. But the prevalence rates deducted by both Priya Datta in a territory care hospital in India and Kanungo et al Department of Microbiology JIPMER, were 12.6% and 20.5% respectively which were found to be lower than the value reported in the study (11,12). But the prevalence rate reported by C. Rodrigues et al was 53% which was found to be higher than the value detected in this study [13].

The prevalence rate of ESBL producing Klebsiella pneumoniae detected in this study was 26.6%. But as per the studies of Hansotia et al in 1997, the prevalence of ESBL producing Klebsiella isolates was detected as 76.5% (14) in central India which was found to be higher than this study. Kanungo et al [12] also reported higher prevalence of ESBL producing Klebsiella isolates was 43.75%. The results of foreign studies have been studied by Subha et al [15]. The incidence of ESBL producing Klebsiella isolates in the United States has been reported to the 5%. In France and England – 14 to 16% ESBL producing Klebsiella isolates have been reported. In a report from France, 24.8% ESBL positive Klebsiella pnemoniae were found in patients of more than 16 year of age. This shows that there is a wide disparity between Indian studies and Foreign studies.

In this study a prevalence of ESBL producing Escherichia coli was identified as 33.3%. But Kanungo et al [12] had reported incidence of 58.06% which was higher than the value of this study. Sensitivity and Specificity of ESBL detection test – while referring the studies of Priya Datta it was found that a comparison of three tests - DDST, 3 Dimensional Test (3D) and IPT was made for the screening of ESBL strains [11]. In their study IPT was found to be best screening method when combined with use of a ceftriaxone disk. IPT detected the maximum number of ESBL producing strains. Ceftriaxone detected the maximum ESBL rate in DDS, 3D and IPT followed by cefotaxine and ceftazidime [11]. Courdron et al found the sensitivity of ceftriaxone to be 88% ceftazidime 79% for these ESBL screening methods. Further it was confirmed by Hoe et al studies which has reported IPT was 100% sensitive and DDS was 96% sensitive [11].

When analyzing the sensitivity of DDST it was found that Thomson Sandars has reported DDS to be 79% sensitive, Vercauteren et al [16] found the sensitivity to be 93% and Shukla et al reported 90.6% sensitive, which were co-related with this study. Some studies questioned the sensitivity of Double Disk Test. Several modifications have been recommended including changing the distance between the Disks. A distance of 20mm center to center has been recommended by Priya Datta, Archana Thakkur in their study [11]. A distance of 30 mm from center to center has been recommended by U. Chaudhry, R. Agarwal [18].

4. Conclusion

From this study we conclude that gram negative bacilli are common etiological agents of Surgical Site infections. Emergence of Beta lactamase producers like *Pseudomonas, Escherichia, Klebsiella spp*, added severity of the condition. ESBL production was found to be coexsisted with resistance to several other antibiotics. ESBLs are encoded by plasmids which also carry co- resistant genes for other antibiotics. Impact of post discharge surveillance of surgical infections in operative surgery is a very important factor. Surveillance feedback to surgeons is important in reducing Surgical Site Infections (SSIs) post operative infection will be missed unless post discharge surveillance is undertaken. To reduce the wound sepsis rate as post operative wound closure (primary closure) during contaminated operations has been associated with nearly 40% wound sepsis rate based on Knightons wound severity index. (Knighton et al) and post surgical mortality rate, proper deduction and timely reporting of presence of ESBL producing GNB in

post operative wounds is very essential. It is suggested that routine diagnosis of ESBL producing strains in post operative wound infections has to be carried out inorder to avoid undesired effects of multi-drug resistant ESBL producing Klebsiella by strict control of antibiotic usage. The restricted use of antibiotics will lead to the withdrawal of selective pressure and resistant bacteria will no longer survive in such settings. These findings were the net result of this study. In addition, an effective national and state level antibiotic policy and draft guidelines should be introduced to preserve the effectiveness of antibiotics and for better patient management.

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6. References:

1. Barbara MS, Mark TL. Nosocomial infections: An overview. In: Barbara JH, editor. Clinical and pathogenic microbiology. 2nd ed. St louis: Mosby; **1994**. p. 83-99.
2. Fatima Numanovic, Mirsada Hukic, Zineta Delibegovic, Nijaz Tihic, Selma Pasic, Merima Gegic - Comparison of double disk synergy test, VITEK 2 and Check- MDR CT102 for detection of ESBL producing isolates**;** Acta Medica Academica **2013**, 42(1): 15-24
3. Rubeena Hafeez, Maleeha Aslam, Farzana Mir, M. Tahir Iffat Javaid And Aasma N. Ajmal; Frequency Of Extended Spectrum Beta Lactamase Producing Gram Negative Bacilli Among Clinical Isolates ; Biomedica Vol.25, Jul. – Dec. **2009**/Bio-19.Doc P. 112 – 115
4. Modi Dhara, Patel Disha, Patel Sachin, Jain Manisha, Bhatt Seema, Vegad M M; Comparison Of Various Methods For The Detection Of Extended Spectrum Beta Lactamase In Klebsiella pneumoniae Isolated From Neonatal Intensive Care Unit, Ahmedabad; National Journal Of Medical Research; Volume 2, Issue 3, July – Sept **2012**, pp 348-353.
5. Horan TC, Gaynes RP, Martone WJ. CDC definitions of surgical site infections 1992: A modification of CDC definitions of surgical wound infections. *Am J Infect Control.* **1992**, 20: 271.
6. National Academy of Sciences, National Research Council, Division of Medical sciences, Ad Hoc Committee on Trauma: Postoperative wound infections: The influence of ultraviolet irradiation on the operating room and of various other factors. *Ann Surg*, **1964**, 160 (Supp12): 1.
7. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn JC Jr,editors. *Color Atlas and Textbook of Diagnostic Microbiology* 2006;6th ed. 624–662. Philadelphia: Lippincott Raven.
8. Mackie & McCartney Practical Medical Microbiology; 14th Edition; Elsevier Publications. Chapter 7 – Tests For Identification of Bacteria, Pp 131 – 150
9. Clinical Laboratory Standards Institute-Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement; Supplemental Table 2A-S1. Screening and Confirmatory Tests for ESBLs in *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, and *Proteus mirabilis* for Use With Table 2A**,** M100-S21 ,Vol. 31 No. 1; pg 48-49)
10. Centers for Disease Control and Prevention. National Nosocomial Infections Surveillance (NNIS) report, data summary from October1986-April 1997. *Am J Infect Control* **1997**, 25: 477-87.
11. Priya Datta, Archana Thakkur – Jpn.J.Infect Dis.57, 146-149 2004 prevalence of clinical strains resistant to various beta lactams in a Terittery Care Hospital in India.
12. Reba Kanungo. Aswin. A. Anantha Kumar. A. Kumar and S. Badrinath. Detection of ESBL producers – amond surgical wound infections and burns patients in JIPMER. Indian Journal of Medical Microbiology, **2000,** 18(4): 160-165.
13. C. Rodrigues, P. Joshi, S.H. Jani, M. Alphonse, R. Radhakrishnan, A. Mehta. Detection of B lactamases in Nosocomial Gram Negative Clinical Isolates. Indian Journal of Medical Microbiology **2004,** 22(4) : 247 – 250
14. Jerestin B. Hansotia, Vandana Agarwal, A.A. Pattak and A.M. Saojii. ESBL
15. mediated resistance to third generation cephalosporins in Kleb pneumoniae in Nagpur.Central India Indian J. Med. Res 105 April **1997,** pp.158-161.
16. A. Subha, S. Anandham B.s.V. Alavandi. ESBL Production and Multi Drug Resistance in Kleb Species isolated from children under five with intestinal and extra intestinal infections.
17. Indian Journal Medi Res 113 May **2001**, 181
18. 16.Vercauteren E et al. Comparision of screening methods for detection of ESBL and their prevalence among blood isolates of Escherichia and Klebsiella species in a belgian teaching hospital.Journal of Clinicla Microbiology.Sep **1997**, 35(9): 2191-2197
19. 17.Shukla. I, Tiwari. R, Agarwal. M. Dept. of Microbiology, J.N. Medical College, Aligarh University, Aligarh–Prevalence of ESBL producing Klebsiella pneumoniae in a Tertiary Care Hospital : year **2004**, 22(2) : 87-91

20. U.Chaudhary, R. Aggarwal, ESBL – An emerging threat to clinical therapeutics. *Indian Journal of Medical Microbiology*. **2004,** 22 (2) : 75 – 80