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Bacterial Profile of Surgical Site Infections and ESBL Detection among the Gram Negative Isolates

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Abstract **Original Research Article**

Purpose –Surgical site infection is one of the common post-operative complications. Microorganisms with emerging drug resistance being one of the major contributing factors, extended spectrum beta lactamase (ESBL) producing Gram Negative Bacilli pose a challenge in the treatment of surgical site infections. The present study is undertaken to study the prevalence of the ESBL production among various Gram Negative Bacilli isolated from surgical site infections at a tertiary care hospital. ***Material & Methods***: A cross-sectional study during May 2018 to December 2018 including 112 samples from infected surgical wounds. A total of 73 bacterial isolates were identified by standard microbiological identification tests and Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method following CLSI guidelines 2018. Gram negative isolates which were resistant to third generation cephalosporins were screened for ESBL production by double disc diffusion method and confirmation was done by Combination disc method. ***Results:*** Among 112 samples from infected surgical wounds, 73 (65.17%) were culture positive with 42 (57.53%) Gram positive isolates and 31(42.46%) Gram negative isolates. Gram positive isolates included 32 Staphylococcus aureus and 10 Coagulase negative staphylococci (CoNS). Gram negative bacilli included 14 Klebsiella species, 10 Escherichia coli, 4 Pseudomonas aeruginosa, 2 Proteus species and 1 Citrobacter freundii. ESBL production was detected in 18 (58.06%) Gram negative bacilli by Double disc diffusion method and confirmed by Combination disc method in 12 (38.7%) Gram negative bacterial isolates. ***Conclusion:*** A significant number of ESBL producing Gram negative bacteria were detected from surgical site infections which indicate the necessity of developing and implementing antibiotic policy.

Keywords: Extended spectrum beta lactamases, surgical site infection, Gram negative bacteria, and antibiotic susceptibility.

INTRODUCTION

Surgical site infections are second most common Hospital associated infections and common post-operative complication [1]. Emerging drug resistance being one of the major contributing factors, surgical site infections are important causes of mortality and morbidity and result in increased hospital stay [2]. Beta lactam antibiotics are among the most irrationally used antibiotics worldwide and the emergence of resistance to these drugs has resulted in clinical crisis [2].

Aim

To study the bacteria causing surgical site infections and identify the extended spectrum beta lactamase production among various Gram Negative bacteria causing Surgical site infections.

MATERIALS & METHODS

The Present study was carried out in the Department of Microbiology, Osmania general hospital, Hyderabad for a period of 8 months (May 2018-December 2018) after clearance from institutional ethical committee.112 Pus and wound swab specimens collected aseptically from clinically suspected surgical site infections were included in the study. They were processed by Gram staining and Aerobic culture on Blood agar and MacConkey agar. After incubation at 37^0C for 24hours the isolates were identified by standard microbiological identification tests [3]. Antimicrobial susceptibility testing of isolates was performed on Muller Hinton agar by Kirby Bauer disc diffusion method following CLSI guidelines 2018 [4]. While tesing Gram negative isolates indicator third generation cephalosporins (Cefotaxime and Ceftazidime) were included to screen for ESBL production. Isolates resistant to both indicator cephalosporins were subjected to confirmatory test to demonstrate synergy using combination disc method, Cefotaxime (30µg) and Cefotaxime + Clavulanic acid (30/10) placed 30mm apart from center to center and >5mm increase in zone diameter for Cefotaxime + Clavulanic acid and the distorted zone around cefotaxime disc facing Cefotaxime -Clavulanic acid disc is considered as confirmed ESBL producer [5]. (Diagram -1)

RESULTS

A total of 112 samples from infected surgical wounds were included in the study, 73 (65.17%) were culture positive and 39 (34.83%) were culture negative.

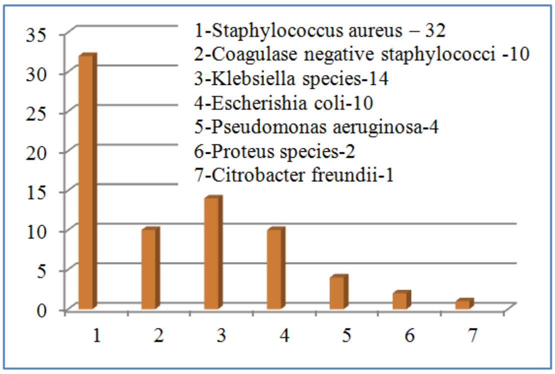

Diagram-1: Bar Diagram showing various culture isolates (n =73)

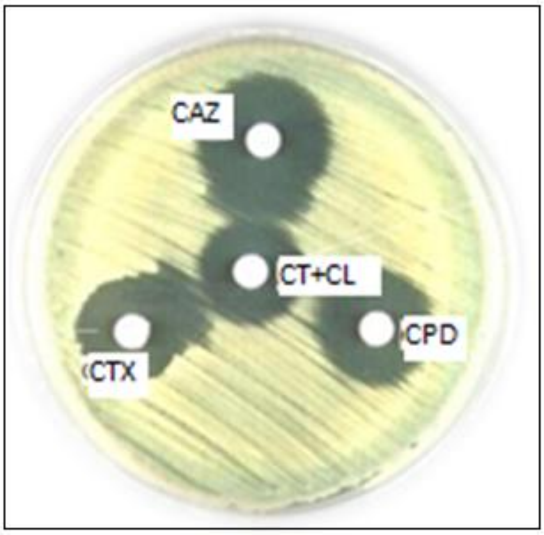

Diagram-2: Combination Disc Method

Among 73 culture isolates 42(57.53%) were Gram positive isolates and 31(42.46%) were Gram negative isolates. Gram positive isolates included Staphylococcus aureus 32 (76.41%) and Co NS 10 (23.81%). Among the 31 Gram negative bacilli Klebsiella species were 14 (45.16%) followed by Escherishia coli 10 (32.25%), Pseudomonas aeruginosa 4 (12.9%), Proteus species 2 (6.45%) and Citrobacter freundii 1 (3.22%) as shown in Diagram 2.

Table-1: Antimicrobial susceptibility pattern of Gram positive bacterial isolates (n=42)

ANTIBIOTIC	Staphylococcus aureus (n=32)		CoNS (n=10)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Ampicillin	4(12.25)	28 (87.5)	2(20)	8 (80)
Amoxycillin-Clavulanic acid	26(81.25)	6(18.75)	8 (80)	2 (20)
Ciprofloxacin	28 (87.5)	4(12.5)	9 (90)	1 (10)
Gentamicin	27 (84.4)	5 (15.6)	9 (90)	1 (10)
Cefotaxime	12 (37.5)	20 (62.5)	4 (40)	6 (60)
Cefoperazone	11 (34.4)	21 (65.6)	4 (40)	6 (60)
Linezolid	32 (100)	0(0)	10(100)	0 (0)

All the Gram positive bacterial isolates showed 100% susceptibility to Linezolid. Staphylococcus aureus isolates showed 87.5% susceptibility to Ciprofloxacin, 84.4% susceptibility to Gentamicin, 81.25% susceptibility to Amoxycillin-Clavulanic acid. Suscetibility to cephalosporins was less showing 37.5% susceptibility to Cefotaxime, 34.4% sensitivity to Cefoperazone, Among CoNS 90% susceptibility was observed to Ciprofloxacin and Gentamicin followed by 80% susceptibility to Amoxycillin-Clavulanic acid, susceptibility to Cephalosporins was only 40%. (Table 1)

All the Gram negative bacterial isolates showed 100% sensitivity to Carbapenems (Imepenem, Meropenem) and Piperacillin +Tazobactum except Escherichia coli which showed 90% susceptibility to Piperacillin + Tazobactum.Susceptibilty to Amoxycillin + Clavulanic acid was varied among Gram negative bacterial isolates showing 71.4% susceptibility among Klebsiella isolates, 60% susceptibility among Escherichia coli, 100% susceptibility among Citrobacter freundii. Klebsiella species showed 35.7% susceptibility to Cephalosporins (Ceftazidime, Cefotaxime, and Ceftriaxone). 75% of Pseudomonas aeruginosa isolates were susceptible to Amikacin and Ceftazidime. (Table 2)

Table-2: Antimicrobial susceptibility pattern of Gram negative bacteria (n =31)

ANTIBIOTIC	Klebsiellasps. (n=14)		Escherichia coli(n=10)		Pseudomonas aeruginosa(n=4)		Proteus sps(n=2)		Citrobacter freundii (n=1)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive(%)	Resistant(%)	Sensitive (%)	Resistant(%)
Amikacin	12(85.7)	2(14.3)	8(80)	2(20)	3(75)	1(25)	2(100)	0(0)	0(0)	1(100)
Ceftriaxone	5(35.7)	9(64.3)	4(40)	6(60)	NT	NT	2(100)	0(0)	1(100)	0(0)
Ciprofloxacin	11(78.5)	3(21.5)	6(60)	4(40)	2(50)	2(50)	1(50)	1(50)	0(0)	1(100)
Cotrimoxazole	5(35.7)	9(64.3)	4(40)	6(60)	NT	NT	1(50)	1(50)	0(0)	1(100)
Amoxycillin	3(21.5)	11(78.5)	1(10)	9(90)	NT	NT	NT	NT	0(0)	1(100)
Amoxycillin + Clavulanic acid	10(71.4)	4(28.6)	6(60)	4(40)	NT	NT	NT	NT	1(100)	0(0)
Piperacillin+Tazobactum	14(100)	0(0)	9(90)	1(10)	4(100)	0(0)	2(100)	0(0)	1(100)	0(0)
Cefotaxime	5(35.7)	9 (64.3)	4(40)	6(60)	2(50)	2(50)	2(100)	0(0)	1(100)	0(0)
Ceftazidime	5(35.7)	9 (64.3)	4(40)	6(60)	3(75)	1(25)	2(100)	0(0)	1(100)	0(0)
Imipenem	14(100)	0(0)	10(100)	0(0)	4(100)	0(0)	2(100)	0(0)	1(100)	0(0)
Meropenem	14(100)	0(0)	10(100)	0(0)	4(100)	0(0)	2(100)	0(0)	1(100)	0(0)

ESBL production detected in 18(58.06%) Gram negative bacterial isolates using double disc diffusion test which included Klebsiella species 9(64.3%), Escherichia coli 7(70%), Pseudomonas aeruginosa 2(50%).

Table-3: Detection of ESBL production among various Gram negative isolates (n =31)

Isolate	Total Number	ESBL detection by Double disc Diffusion method (%)	ESBL confirmed by Combination disc method (%)	ESBL Non-producers (%)
Klebsiella species	14	9(64.3%)	7(50%)	7(50%)
Escherishia coli	10	7(70%)	4(40%)	6(60%)
Pseudomonas aeruginosa	4	2(50%)	1(25%)	3(75%)
Proteus species	2	0(0%)	0(0%)	0(0%)
Citrobacter species	1	0(0%)	0(0%)	0(0%)
Total	31	18(58.06%)	12(38.7%)	19(61.3%)

ESBL production was confirmed by Combination disc method in 12 (38.7%) Gram negative bacterial isolates. 50% of Klebsiella isolates, 40% of Escherichia coli isolates, 25% of Pseudomonas aeruginosa isolates were confirmed ESBL producers (Table 3).

All the ESBL producing Gram negative bacterial isolates were sensitive to Imipenem. 83.3% were sensitive to Piperacillin+Tazobactum, 33.4% were sensitive to Ciprofloxacin, 16.7% were sensitive to Cotrimoxazole and 8.33% sensitive to Amikacin.

DISCUSSION

Surgical site infections lead to prolonged hospital stay and act as source in the spread of Hospital infections. Culture positivity in the present study was 65.17% and it varied in the previous studies between 30.8% and 91% and it coincided with the findings of V. Rambabu *et al*. [2]. The percentage of Gram positive bacterial isolation was 57.53% in the present study and was higher when compared to the previous studies as shown in Table 4. The commonest bacterial isolate was Staphylococcus aureus 32 (43.83%) from various SSIs and it coincided with previous studies except Islam et al which showed Escherishia coli as most common isolate [Table 4].

Among the 31 Gram Negative bacterial isolates Klebsiella species 14 (45.16%) was the most common in the present study followed by Escherishia coli 10 (32.25%).In previous studies of Islam *et al.* [8],Chada CKR *et al.* [1], Mundhadha and tempe [9] Escherishia coli was the most common isolate followed by Klebsiella species. (Table 4)

Table-4: Comparison of Percentage of Bacterial isolates and ESBL production in Gram Negative Isolates with various studies

S. No	Study	Percentage of bacterial isolation	Most common Gram Positive isolate	Most common Gram Negative isolate	% of ESBL producers among Gram negative isolates
1	Kownhar H *et al*. 2003 [7]	30.8%	Staphylococcus aureus 37%	Pseudomonas aeruginosa 37%	--
2	Mundhada AS, Tempe S, 2011 [9]	32%	Staphylococcus aureus 29.16%	Escherichia coli 20.83%	--
3	Islam *et al*. 2014 [8]	73.9%	Staphylococcus aureus 22.1%	Escherichia coli 29.4%	30.19%
4	V.Rambabu *et al*. 2015[2]	63.3%	Staphylococcus aureus 20.31%	Pseudomonas aeruginosa 18.42%	27.63%
5	Chada CKR *et al*. 2017 [1]	91%	Staphylococcus aureus 25.34%	Pseudomonas aeruginosa 17.19% & Esch. coli 17.19%	11.64%
6	Present study, 2018	65.17%	Staphylococcus aureus 43.83%	Klebsiella sps 19.17%	38.7%

In the present study ESBL incidence was 38.7% among all Gram negative bacterial isolates where as in other studies the incidence varied from 11.64% and 30.19%. (Table 4)

CONCLUSION

Post-operative surgical site infections with antibiotic resistant organisms are common cause for non-healing wound and prolonged hospital stay.

Continuous analysis of bacterial isolates and their antimicrobial resistance pattern helps in developing antibiotic policy to rationalize the use of antibiotics and also helps in control of nosocomial spread.

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