International Journal of Medical Research Professionals
P-ISSN: 2454-6356; E-ISSN: 2454-6364
DOI: 10.21276/ijmrp

IJMRP

Original Research Article.

Study of Antibiogram of Gram-Negative Bacterial Isolates From Surgical Wound Site Infection

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ABSTRACT

Aim: Study of antibiogram of gram-negative bacterial isolates from surgical wound site infection.

Method: Samples were collected from post-operative wards of general surgery department of Mahatma Gandhi medical college & Hospital, Jaipur.

All wound samples received in the Microbiology laboratory from the surgery department were cultured on Blood and MacConkey's agar and identification and susceptibility of the implicating pathogen was done using Vitek 2 automated/Manual method as per CLSI guidelines. Detection of MBL cases were done by double disc synergy test and confirmed by E-test of Meropenem.

Results: Total 250 non-repetitive variable samples from various wards were collected and processed in MGMCH, Jaipur. Out of 250, 88 (35%) samples where found growth of microbial pathogens, rest were sterile. Out of 88 cultured samples, 72 were reported as gram negative isolates. In GNB isolates- 32 were E.coli, 16 were pseudomonas, 12 were klebsiella, 9 Acinetobacter and 3 proteus were found. Out of 72 GNB isolates, 51 (70.8%) samples were found CRGNB rest were sensitive to carbapenems.

Conclusion: Choosing the best agent for the treatment of infections caused by these pathogens is one of the most important challenges facing practitioners. Regular monitoring and documentation of carbapenem resistance should be done. Colistin could be a drug of choice in carbapenem resistant gram-negative bacilli infections.

Key Words: Surgical Site Infection, GNB, Carbapenem Resistance.

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Article History:

Received: 02-06-2020, **Revised:** 28-06-2020, **Accepted:** 14-07-2020

Access this article online	
Website: www.ijmrp.com	Quick Response code
DOI: 10.21276/ijmrp.2020.6.4.025	

INTRODUCTION

Antibiotic resistance is now becoming a major concern of global threat. It is more in developing countries where infectious diseases are still growing. In the developing countries, there is irrational use of antibiotics mainly because, due to limited resources, many clinicians opts symptomatic treatment.[1,2] In addition there is an extensive over the counter treatment with widespread self-medication and incomplete course of antibiotics. These are well known factors that facilitate development of antibiotic resistance.

Infection is come up by most of the surgeons, as surgeries invariably impair the first line of host defenses between environmental microbes and the host's internal tissue resulting in postoperative wound infection known as surgical site infections (SSI).[1]

75% of death of the patients with hospital acquired infections was reported to be related to postoperative infections. The number of surgical patients in developing countries is also increasing but surgical care given to the patients is poor. Various factors affecting the infection rate include skin preparation, wound contamination, the length of hospital stay, drainage of wounds, age and duration of surgery. Success in surgery depends on prevention and proper management of a wound. In order to adapt to the policies which, decrease the incidence of SSIs, the most important requirement is to collect data, perform wound surveillance and surgical inspection.[2,3]

The aim of the present study is to find incidence and risk factors for SSI in the General Surgery department of our hospital and to know bacteriological profile and antibiogram of organisms causing SSIs

MATERIALS AND METHODS

Samples were collected from post-operative wards of general surgery department of Mahatma Gandhi medical college & Hospital, Jaipur.

Clinical swabs from non-healing ulcers, pus/wound swab & other samples from sterile body fluids were collected by taking aseptic precautions. The clinical samples were subjected to direct microscopy by Gram stain and further were cultured on blood agar, MacConkey's agar & Thioglycolate broth. A culture plates were incubated overnight at 37ºC. Isolated gram-negative organisms were further identified by standard set of biochemical tests.[8]

Those plates showing no growth was further incubated for another 24 hrs, identification and susceptibility of the implicating pathogen was done using Vitek 2 automated/Manual method as per CLSI guidelines.[9]

Antibiotic Susceptibility Testing (Anti-biogram): Antimicrobial sensitivity testing was performed on Mueller Hinton agar (Hi-Media, Mumbai) plates by disk diffusion method according to CLSI guidelines.[9] The diameter of the zones of inhibition on MHA was interpreted as sensitive, intermediate and resistant. *Escherichia coli* ATCC 25922 (β-lactamase negative) *Pseudomonas aeruginosa* ATCC 27853 (β- lactamase negative) and *Klebsiella pneumoniae* ATCC 700603 (ESBL positive) strains were used as control organisms. Organism with intermediate levels of resistance to the antibiotics Meropenem & Imipenem were included in percentage of resistant organisms for final analysis .by Imipenem & EDTA combined disc Imipenem test.[5-7]

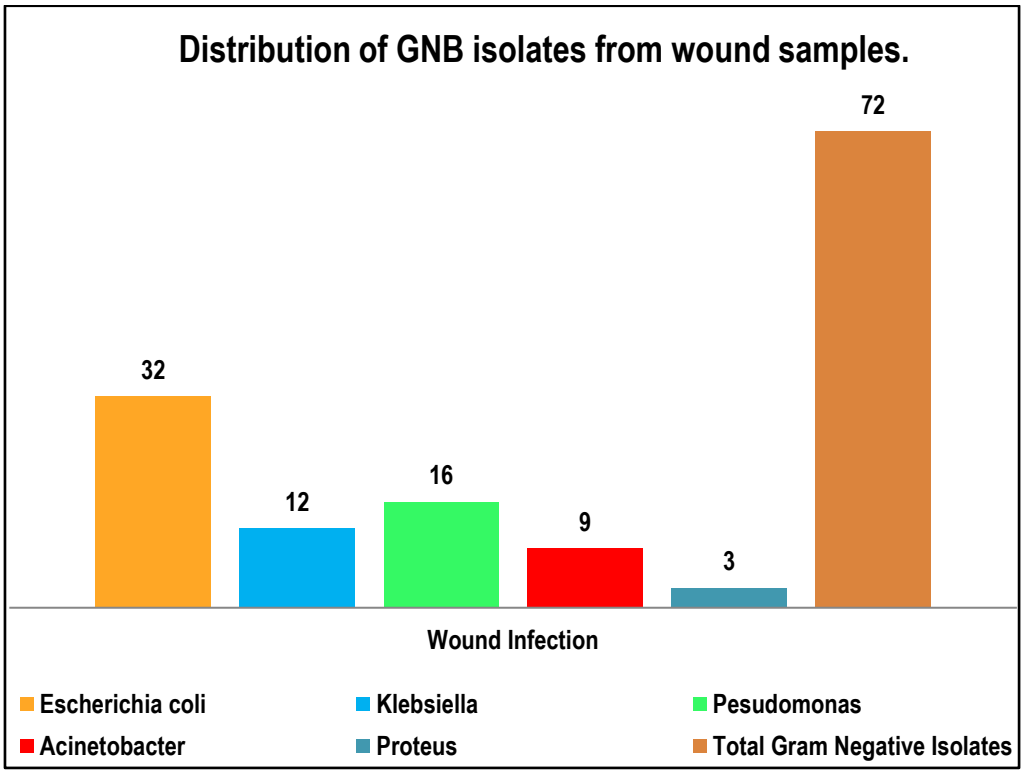

Fig 1: GNB isolates from wound samples.

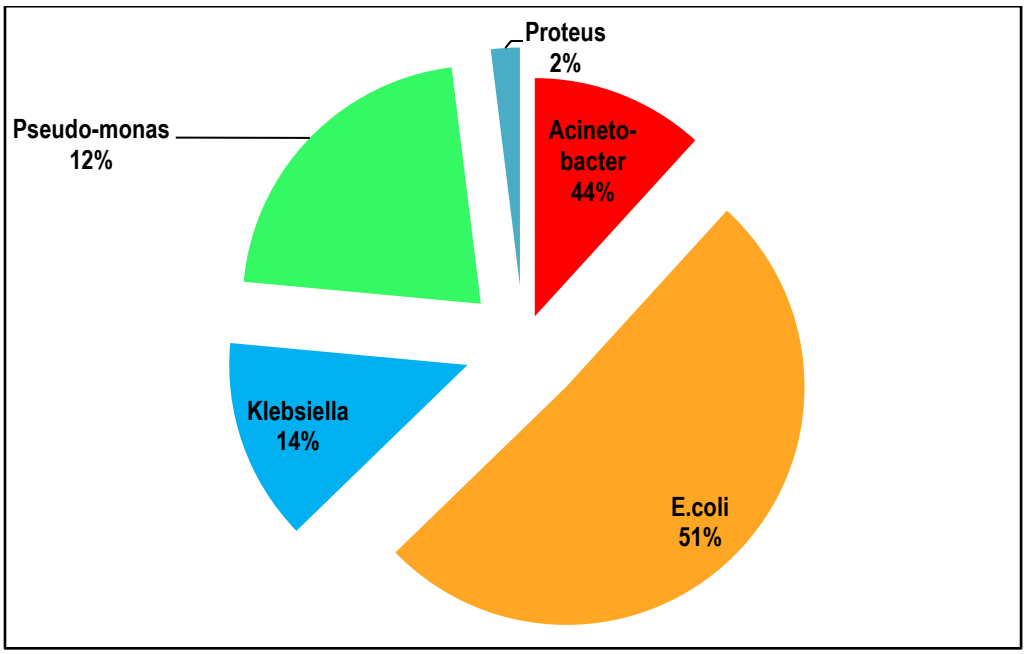

Fig 2: Distribution of CRGNB isolates

Table 1: Antibiotic sensitivity pattern of CRGNB wound isolates.

Antibiotic Testing	E.coli n= 32	Pseudomonas n=16	Klebsiella n= 12	Acinetobacter n=9	Proteus n=3	Sensitivity %
CI	32	16	12	8	2	**97%**
Pi	2	1	2	5	1	15%
PiT	2	3	2	5	1	18%
OF	2	0	5	0	0	10%
CAZ	1	0	2	0	0	4%
MRP	6	2	3	1	0	17%
IMP	5	2	3	1	0	15%
ETP	1	-	0	-	0	1.39%
DO	2	0	1	1	-	6%
GEN	0	0	0	1	-	1.39%
COT	1	0	0	0	1	3%
AT	1	-	0	-	-	1.39%
A/S	3	1	2	22	1	40.27%
CPM	1	1	4	0	0	8%
TGC	6	-	10	16	-	**44.44%**
LE	1	-	3	8	1	18.06%
AK	1	2	3	0	1	10%
PB	-	6	-	0	-	8.33%
CB	-	4	-	19	-	32%
TOB	-	2	-	3	-	6.94%

CI – Colistin, Pi – Piperacillin, PiT- Piperacillin-Tazobactum, OF- Ofloxacin, CAZ- Ceftazidime, MRP- Meropenem, IMP- Imipenem, ETP- Ertapenem, DO-Doxycycline, GEN- Gentamycin, COT- Cotrimoxazole, AT- Aztreonam, A/S- Ampicillin-Sulbactum, CPM- Cefepime, TGC-Tigecycline, LE- Levofloxacin, AK- Amikacin, PB- Polymyxin B, CB- Carbenicillin, TOB-Tobramycin, FO-Fosfomycin, NIT- Nitrofurantoin

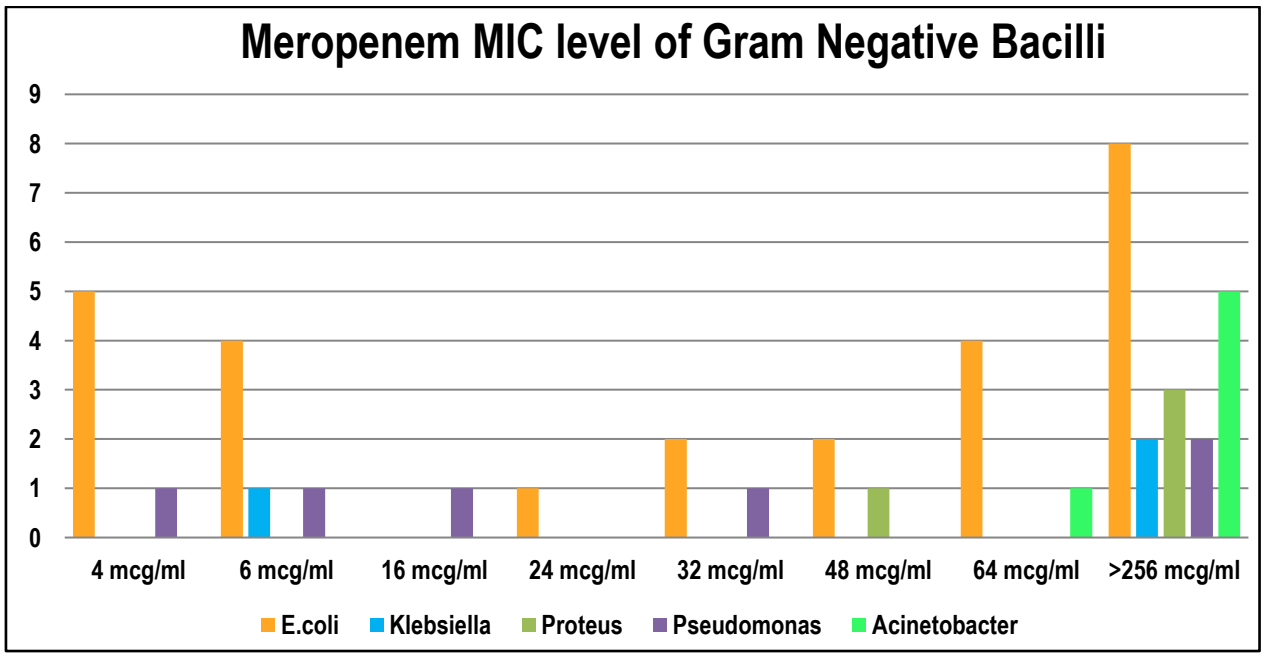

Fig 3: MIC level of Meropenem in Gram Negative bacilli from Surgical Site Infection

RESULTS

Total 250 non-repetitive variable samples from post-operative ward of surgery department were collected and processed in Mahatma Gandhi Medical College and Hospital, Jaipur. Out of 250, 88 (35%) samples where found growth of microbial pathogens, rest were sterile. Out of 88 cultured samples, 72 were reported as gram negative isolates. In GNB isolates- 32 were E.coli, 16 were pseudomonas, 12 were klebsiella, 9 Acinetobacter and 3 proteus were found.[Fig.1]

Out of 72 GNB isolates, 51 (70.8%) samples were found CRGNB (carbapenem Resistant Gram-Negative Bacilli) rest were sensitive to carbapenems [Fig.2]. In 51 CRGNB samples 38 were obtained from males and 13 from females. Maximum 12 cases were in between the age of 41-50 years & mean of the age was 46.9 ±20.53.

Various antibiotics included in the study were sourced from commercial batches belonging to β-lactam, aminoglycoside,

quinolone, and tetracycline classes as per the CLSI guideline. Carbapenem resistant organisms were not only resistant to carbapenem group but also resist to most of antibiotics. Colistin is only drug which showed 97% sensitivity in all CRGNB isolates. [Table 1] Total 45 cases were MBL reported out of 51 CRGNB by Meropenem with & without EDTA Ezy MIC™ Strips. Maximum resistance was found in member of *E.coli*. [Fig. 3]

DISCUSSION

The overall carbapenem resistance in the present study was 28.8%. The carbapenem resistance rate among GNB varies widely in the literature. Taneja et al. reported it was 36.4%, Gladstone et al. were found 12.2% while Gupta et al mentioned it 17.32% and Dutta et al documented it as 7.87%.[3,11] The incidence varies from as low as 1.8% to over 30% in India.[12,13]

In our study among, 35 (14%) entoerbacteraceae were resistant to carbapenems. This resistance rate was compared with several studies done in India. Many authors have used one or more carbapenems as indicator drug for testing resistance to carbapenems by disc diffusion or MIC method. Resistance to carbapenems ranged from 2% to 22% in Indian studies. Gupta et al reported less carbapenem resistance rate 3.61%[12] while Gladstone et al found 12.2%, wattal et al 13-51%, Datta et al 17-22% and Dardi kaur et al reported 8.33%.[5,14,15]

In the present study, out of 16 NFGNB 56 (6.4%) were resistant to carbapenems. In Indian studies, carbapenem resistance in *P. aeruginosa* has been reported from centres in Pondicherry, Vellore, Bangalore, Chandigarh, Mumbai, New Delhi and Varanasi with the rates of resistance between 10.9% and 69%.[16,17] In 83 *Acinetobacter* isolates out of 113 NFGNB 38.93% (44/113) were carbapenem resistant.

Although molecular techniques are regarded as the most appropriate method for the detection of carbapenem resistance, it becomes impractical in a routine diagnostic laboratory setup up due to cost factors, availability of molecular set up.

CONCLUSION

Choosing the best agent for the treatment of infections caused by these pathogens is one of the most important challenges facing practitioners. Regular monitoring and documentation of carbapenem resistance should be done. Colistin could be a drug of choice in carbapenem resistant gram-negative bacilli infections. We also recommend hand hygiene and thorough infection control protocol to prevent the spread of microbes and antibiotic stewardship and tailor-made prophylactic policies based on local susceptibility data should be used.

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Source of Support: Nil. **Conflict of Interest:** None Declared.

Cite this article as: Aditya Mishra, Anil Avasthi. Study of Antibiogram of Gram-Negative Bacterial Isolates From Surgical Wound Site Infection. Int J Med Res Prof. 2020 July; 6(4): 109-12. DOI:10.21276/ijmrp.2020.6.4.025