VOLUME - 13, ISSUE - 03, MARCH - 2024 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper | Clinical Microbiology

SURGICAL SITE INFECTIONS WITH METALLO BETA LACTAMASE PRODUCING ENTEROBACTERIACEAE WITH SPECIAL REFERENCE TO NDM PRODUCING KLEBSIELLA PNEUMONIAE.

Dr Neha Sharma* — Senior Resident , Department Of Microbiology , Government Medical College And Hospital , Nagpur , Maharashtra , India 440003. *Corresponding Author

Dr.(Prof) Sunanda Shrikhande (Zodpey) — Professor And Head , Department Of Microbiology , Government Medical College And Hospital , Nagpur , Maharashtra , India

Dr Priyanka Kalbhor — Assistant Professor ,department Of Microbiology , Government Medical College And Hospital , Nagpur , Maharashtra , India 440003.

ABSTRACT

Introduction: Surgical site infections (SSI) constitute a major public health problem worldwide and are the second most frequently reported nosocomial infections. They are responsible for increasing the treatment cost, length of hospital stay and significant morbidity and mortality.**Aim:** determine Surgical site infections with Metallo Beta Lactamase producing Enterobacteriaceae with special reference to NDM producing Klebsiella pneumoniae.**Materials and Methods:** Samples were collected using sterile cotton swabs from 241 patients clinically diagnosed of having SSIs and were processed as per standard microbiological techniques. Antimicrobial susceptibility testing was done using modified Kirby-Bauer disc diffusion method. This cross sectional study was conducted for a period of six months (November 2020 to December 2022) in the Department of Microbiology at a tertiary care hospital of central India. **Results:** Out of total 241 samples, 220(91.29%) yielded bacterial growth. Klebsiella pneumoniae (22.27%) was the commonest organism followed by Escherichia coli (18.11%). During the study period, all the 15 MBL producing K. pneumoniae isolates were screened for carbapenemase production on the basis of their reduced susceptibility to Meropenem using Xpert® Carba-R assay. Out of these 15 K. pneumoniae screen positive isolates, 11 were from pus, 4 isolates were from wound swab. All the screening positive isolates (n =15) were found to be positive for blaNDM gene by Xpert® Carba-R assay. Four out of 15 isolates were found to be positive for blaOXA-48 and blaNDM.**Conclusion:** The rate of SSI observed in this study was comparable to other similar studies, however we observed a higher degree of antimicrobial resistance. The emergence of blaNDM-1and OXA-48 harboring Enterobacteriaceae is of concern because such isolates exhibit resistance to drugs commonly used to treat gram-negative infections. Adherence to strict infection control measures, maintenance of proper hand hygiene and optimal preoperative, intraoperative and postoperative patient care will surely reduce the incidence of SSIs.

KEYWORDS : Antimicrobial Resistance ; Infection Control ; Nosocomial Infection ; Surgical Site Infections.

MANUSCRIPT

Surgical Site Infections (SSIs) are one of the most common causes of nosocomial infections and are a common complication associated with surgery, with a reported incidence rates of 2-20%[1]. Surgical Site infections (SSIs) criteria, developed by Centre for Disease Control and Prevention (CDC)[2] defines SSIs as "Infections related to the operative procedure that occur at or near the surgical incision (incisional or organ/space) within 30 days of an operative procedure or within one year if an implant is left in place".

The pathogenesis of SSI has been associated both with the endogenous contamination such as skin flora and exogenous contamination by healthcare personnel or contaminated surgical instruments[3]. Despite the technical advances in infection control and surgical practices, SSI still continue to be a major problem, even in hospitals with most modern facilities[3] . SSI is most important cause responsible for increasing the treatment cost, length of hospital stay and significant morbidity and mortality[4].

Recent years there has been a growing prevalence of Enterobacteriaceae with evolving trends of antimicrobial resistance as a cause of serious infections which is being documented worldwide[5]. *Klebsiella pneumoniae* is known to cause several healthcare-associated infections, including SSIs. The emergence of carbapenem-resistant strains, particularly those harboring NDM and OXA-48 genes, is a growing concern worldwide, with India being one of the epicenter [6,7].

This study aimed to determine Surgical site infections with Metallo Beta Lactamase producing Enterobacteriaceae with special reference to NDM producing Klebsiella pneumoniae.

MATERIAL AND METHODS:

This was a descriptive (Cross-sectional) study conducted in the Department of Microbiology at a tertiary care centre in Central India from November 2020 to December 2022 with approval from institute ethical committee and informed consent was obtained from the subjects.

A total of 241 samples were collected from SSI cases with complaint of pain, swelling, redness, discharge, delayed or non-healing wound and processed as per standard microbiological techniques[8].

Antimicrobial susceptibility testing was performed as per CLSI 2020[9]. Carbapenem resistance genes in *Klebsiella pneumoniae* was detected by using Xpert® Carba-R (real-time PCR) assay[10,11,12]

RESULTS:

Out of 241 samples from SSI cases, 220 (91.29%) were culture positive among which 175 were monomicrobial while 90 organisms were isolated from polymicrobial growth.

Most common isolated organism was *Klebsiella pneumoniae* 59 (22.27%), followed by *E. coli* 48(18.11%), *Proteus mirabilis* 16(6.03%), and *Citrobacter freundii* four (1.50%). 32.08% of organisms were non fermenters and 20.01% were gram positive organisms (Tab.1)

Table 1: Distribution Of Organisms Isolated From Ssi Cases.

Sr. No	Isolated organisms	Total number of isolates (%)
1	*Klebsiella pneumoniae*	59(22.27)

2	*Escherichia coli*	48(18.11)
3	*Pseudomonas aeruginosa*	46(17.36)
4	*Acinetobacter baumannii*	39(14.72)
5	*Staphylococcus aureus*	36(13.59)
6	*Proteus mirabilis*	16(6.03)
7	CoNS	12(4.53)
8	*Enterococcus faecalis*	5(1.89)
9	*Citrobacter freundii*	4(1.50)
	Total	265

CoNS: Coagulase Negative Staphylococcus

Among *Klebsiella pneumoniae* isolates, least resistance 28.81% was found for Imipenem and Meropenem. Among Cephalosporins, Cefepime and Cefoxitin showed less resistance than other Cephalosporins i.e 54.24 % and 45.76 % respectively. (Tab.2)

Table 2: Antimicrobial Resistance Among Enterobacteriaceae Group.

Groups	Antimicrobial agent	Klebsiella pneumoniae n=59 (%)	E. coli n=48 (%)	Proteus mirabilis n=16 (%)	Citrobacter freundii n=4 (%)
Group A	Ampicillin	-#	41(85.42)	12(75)	-#
	Gentamicin	30(50.85)	27(56.25)	8(50)	2(50)
	Tobramycin	27(45.76)	21(43.75)	7(43.75)	1(25)
Group B	Amikacin	24(40.68)	16(33.33)	6(37.5)	1(25)
	Amoxiclav	27(45.76)	21(43.75)	8(50)	-#
	Piperacillin-Tazobactam	26(44.07)	12(25)	3(18.75)	1(25)
	Cefuroxime	46(77.97)	34(70.83)	11(68.75)	-#
	Cefepime	32(54.24)	20(41.67)	8(50)	1(25)
	Cefoxitin	27(45.76)	26(54.17)	6(37.5)	-#
	Cefotaxime	36(61.02)	32(66.67)	9(56.25)	2(50)
	Ciprofloxacin	27(45.76)	22(45.83)	8(50)	1(25)
	Imipenem	17(28.81)	11(22.92)	0	1(25)
	Meropenem	17(28.81)	11(22.92)	0	1(25)
	Piperacillin	46(77.97)	34(70.83)	12(75)	3(75)
Group C	Ceftazidime	36(61.02)	32(66.67)	10(62.5)	2(50)
	Chloramphenicol	27(45.76%)	21(43.75)	10(62.5)	2(50)
	Tetracycline	34(57.63)	29(60.42)	8(50)	1(25)

-#: Intrinsic resistance as per CLSI guidelines

Among *E. coli* isolates, 22.92% were found to be resistant to Imipenem and Meropenem and 25% to Piperacillin tazobactam. Among Cephalosporins; maximum resistance was seen towards Cefuroxime i.e 70.83%, Cefotaxime and Ceftazidime showed resistance to 66.67% isolates, (Tab.3) For *Proteus mirabilis* isolates, all the isolates were sensitive to Imipenem and Meropenem followed by Piperacillin-Tazobactam i.e.18.75 % isolates showed resistance. 37.5% *P.mirabilis* isolates were found to be resistant to Amikacin. Among *Citrobacter freundii,* 1(25%) isolate was resistant to Imipenem and Meropenem. (Tab.2)

Klebsiella pneumoniae was the predominant ESBL producer 19(32.20%) and AmpC producer 6(10.17%) followed by *E.coli*, of which 14 (29.17%) were ESBL and 4(8.33 %) were AmpC producers. Only one isolate of *Proteus mirabilis* was detected as ESBL producer.

Among Enterobacteriaceae, *Klebsiella pneumoniae* 15(25.42%) was predominant MBL producer followed by *E. coli* 9(18.75%) (Fig.4)

During the study period, all the 15 MBL producing *K. pneumoniae* isolates were screened for carbapenemase production on the basis of their reduced susceptibility to Meropenem using Xpert® Carba-R assay. Out of these 15 *K. pneumoniae* screen positive isolates, 11 were from pus, 4 isolates were from wound swab. (Tab.6)

All the screening positive isolates (n =15) were found to be positive for bla_{NDM} gene by Xpert® Carba-R assay. Four out of 15 isolates were found to be positive for bla_{OXA-48} and $bla_{NDM.}$ (Tab.6)

DISCUSSION:

Infection of wounds after surgical operations is a real risk associated with any surgical procedure and represents a significant burden in terms of patient morbidity and mortality. Out of total 265 isolated organisms from culture positive SSI cases, most common organism isolated was *Klebsiella pneumoniae*, 59(22.27%) which is comparable to the study conducted by Verma et al[13] and Anusha et al[14] who reported 13.04% and 19.8% *Klebsiella pneumoniae* respectively.(Tab.1)

Among Enterobacteriaceae group of organisms, all the isolates showed least resistance towards Imipenem, Meropenem followed by Piperacillin-Tazobactam.

In present study, few 17(28.81%) isolates of *Klebsiella pneumoniae* were resistant to Imipenem and Meropenem while 26(44.07%) isolates were resistant to Piperacillin-Tazobactam and 24(40.68%) to Amikacin. Similar findings were reported by Budhani et al[15] who reported maximum sensitivity to Imipenem (76.5%) followed by Piperacillin-Tazobactam (74.6%) respectively. *Klebsiella pneumoniae* isolates in present study has shown less resistance to Cefepime 32(54.24%) and Cefoxitin 27(45.76%) as compared to other cephalosporins. According to study conducted by Negi et al[3] *Klebsiella* species showed highest sensitivity towards Piperacillin and Imipenem and maximum resistance towards Ceftazidime, Ciprofloxacin, Amoxicillin and Gentamicin.(Tab.2)

Among the MBL producing 24 isolates, *Klebsiella pneumoniae* 15(25.42%) was the predominant MBL producer followed by *E.coli* 9(18.75%).This finding is comparable to study, by Sanjay et al[16] who reported 13.22 % of MBL producers among gram negative isolates. (Fig.1)

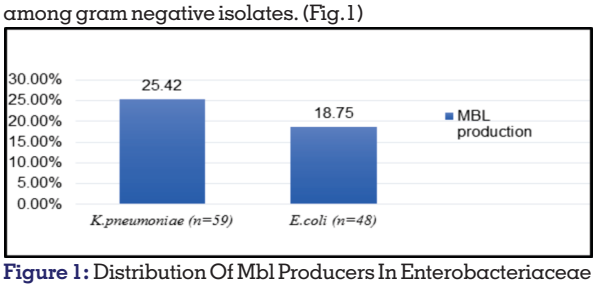

Figure 1: Distribution Of Mbl Producers In Enterobacteriaceae

In the present study, further detection of Carbapenem resistance genes in *Klebsiella pneumoniae* was done by using Xpert Carba-R assay. Out of 15 MBL producing *K. pneumoniae* screen positive isolates, 11 isolates were from pus samples, 4 isolates were from wound swab. All the screening positive isolates (n =15) were found to be positive for bla_{NDM} gene by Xpert Carba-R assay. Four out of 15 isolates were found to be positive for bla_{NDM} and bla_{OXA-48}. (Tab.3)

Table 3. Distribution Of Resistant Genes In Mbl Producing K. Pneumoniae Isolates (N=15)

Total MBL producing K. pneumoniae isolates	bla_{NDM} gene only	Both bla_{NDM} gene and $bla_{OXA-48\ gene}$
15	11	4

Bora et al[17]reported bla_{NDM} in pus samples in *Klebsiella pneumoniae*. Pano-Pardo et al[18] reported bla_{OXA-48} in

Klebsiella pneumoniae causing surgical site infections. There is emerging trend of *K. pneumoniae* isolates co-harbouring bla_{NDM} and bla_{OXA-48} genes[6,7]. The presence of multiple resistance genes and mechanisms was previously reported[19]. However, recent studies shows that dissemination of bla_{NDM} and bla_{OXA-48} among Enterobacteriaceae isolates is mediated by the rapid spread of broad host-range conjugative plasmids[20].

Carbapenem-resistant isolates co-producing multiple carbapenemase genes tend to be extremely high resistant and this leads to limitation in treatment options. These findings may be considered as an alarming threat to the healthcare workers in the hospital settings. The results of our present study therefore confirmed the need for continually and routinely screening for *K. pneumoniae* strains especially those producing bla_{NDM} and bla_{OXA-48} genes to rapidly detect and avoid the dissemination of those strains.

CONCLUSIONS:

Despite the modern aseptic procedures followed in the hospital, SSI remains as a serious problem for patients and surgeons. Rise in prevalence of gram negative organisms showing increase in antibiotic resistance is to be considered as a potential threat in upcoming years. In sight of the increasing incidence of MBL and MDR reported in this study , there is a need for continuous monitoring to determine the resistant pattern of the common isolates.

Carbapenem resistant *K. pneumoniae* have been considered as one of the greatest threats to the global health care. It has been observed that all the screening positive isolates were found to harbour bla NDM gene, and four isolates harbour bla OXA-48 gene. Therefore, the early detection of the resistant gene possessing *K. pneumoniae* isolates with any reduced susceptibility to the carbapenems is indispensable for the choice of the most appropriate antibiotic therapy and the implementation of efficient infection control measures.

The emergence of blaNDM and OXA-48 harboring Enterobacteriaceae is of concern because such isolates exhibit resistance to drugs commonly used to treat gram-negative infections and have shown a propensity to spread rapidly. Therefore, there is the need for active surveillance of carbapenemase-encoding genes as major step to controlling the menance.

Statements and Declaration

Conflict of Interest: The authors declare that they have no conflict of interest.

Institutional Ethical clearance taken before initiation of study.

Informed Consent : All individuals participating have wilfully consented to be a part of study after due informed consent

Financial Declaration : No funding of study of any kind by institution or others.

REFERENCES

1. Hohmann C, Eickhoff C, Radziwill R, Schulz M. Adherence to guidelines for antibiotic prophylaxis in surgery patients in German hospitals: a multicentre evaluation involving pharmacy interns. Infection. 2012;40(2):131-7. https://doi.org/10.1007/s15010-011-0204-7
2. Horan TC, Gaynes RP, Martogne WJ, Jarvis WR, Emori TG. CDC definitions of nosocomial surgical site infections, 1992: a modification of CDC definitions of surical wound infections. Infection Control & Hospital Epidemiology. 1992;13(10):606-8. https://doi.org/10.2307/30148464
3. Negi V, Pal S, Juyal D, Sharma MK, Sharma N. Bacteriological profile of surgical site infections and their antibiogram: A study from resource constrained rural setting of Uttarakhand state, India. Journal of clinical and diagnostic research: JCDR. 2015;9(10):17.https://doi.org/10.7860/JCDR/2015/15342.6698
4. Ahmed MI. Prevalence of nosocomial wound infection among postoperative patients and antibiotics patterns at teaching hospital in Sudan. North American journal of medical sciences. 2012;4(1):29. https://doi.org/10.4103/1947-2714.92900
5. Kasukurthy L, Bathala M. Bacteriological profile of Surgical Site Infections (SSIs)-a study in a tertiary care hospital. J Evid Based Med Heal. 2020;7(32):1612-6. http://doi.org/10.18410/jebmh/2020/338
6. El-Domany RA, El-Banna T, Sonbol F, Abu-Sayedahmed SH. Co-existence of NDM-1 and OXA-48 genes in Carbapenem Resistant Klebsiella pneumoniae clinical isolates in Kafrelsheikh, Egypt. African Health Sciences. 2021;21(2):489-96. https://doi.org/10.4314/ahs.v21i2.2
7. El-Kholy AA, Elanany MG, Sherif MM, Gad MA. High prevalence of VIM, KPC, and NDM expression among surgical site infection pathogens in patients having emergency surgery. Surgical Infections. 2018;19(6):629-33. https://doi.org/10.1089/sur.2018.088
8. Collee J, Duguid J, Fraser A, Marmion B, Simmons A. Specimen Collection and Transport Mackie and Mc Cartney Practical Medical Microbiology. 2006:95-111. https://doi.org/10.1089/sur.2018.088
9. CLSI. Clinical and Laboratory Standards Institute, M100 Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. USA: CLSI Wayne, PA; 2020.
10. Lafeuille E, Laouira S, Sougakoff W, Soulier-Escrihuela O, Leconte J, Garrec H, et al. Detection of OXA-48-like carbapenemase genes by the Xpert® Carba-R test: room for improvement. International journal of antimicrobial agents. 2015;45(4):441-2. https://doi.org/10.1016/j.ijantimicag.2014.12.009
11. Anandan S, Damodaran S, Gopi R, Bakthavatchalam YD, Veeraraghavan B. Rapid screening for carbapenem resistant organisms: current results and future approaches. Journal of clinical and diagnostic research: JCDR. 2015;9(9):DM01. https://doi.org/10.7860%2FJCDR%2F2015%2F14246.6530
12. Carba-R X. Revised Guidelines July 2020 , Xpert® Carba-R Assay. Sunnyvale , CA, USA Cepheid 2020; 2020.
13. Verma AK, Kapoor A, Bhargava A. Antimicrobial susceptibility pattern of bacterial isolates from surgical wound infections in tertiary care hospital in Allahabad, India. Internet Journal of Medical Update-e journal 2012;7(1).
14. Anusha S, Vijaya L, Pallavi K, Manna P, Mohanta G, Manavalan R. An Epidemiological study of surgical wound infections in a surgical unit of tertiary care teaching hospital. Indian Journal of Pharmacy Practice. 2010;3(4).
15. Budhani D, Kumar S, Sayal P, Singh S. Bacteriological profile and antibiogram of surgical site infection/postoperative wound infection. IJMRR. 2016;4(11):1994-9. https://doi.org/ 10.17511/ijmrr. 2016.i11.17
16. Sanjay K, Prasad MN, Vijaykumar G. A study on isolation and detection of drug resistance gram negative bacilli with special importance to post operative wound infection. J Microbiol Antimicrob. 2010;2(6):68-75. https://doi.org/
17. Bora A, AhMed G. Detection of NDM-1 in clinical isolates of Klebsiella pneumoniae from Northeast India. J Clin Diagn Res. 2012;6(5):794-800. https://doi.org/jcdr/2012/4385:2192
18. Pano-Pardo JR, Ruiz-Carrascoso G, Navarro-San Francisco C, Gómez-Gil R, Mora-Rillo M, Romero-Gómez MP, et al. Infections caused by OXA-48-producing Klebsiella pneumoniae in a tertiary hospital in Spain in the setting of a prolonged, hospital-wide outbreak. Journal of Antimicrobial Chemotherapy. 2013;68(1):89-96. https://doi.org/10.1093/jac/dks364
19. Vanegas JM, Cienfuegos AV, Ocampo AM, López L, del Corral H, Roncancio G, et al. Similar frequencies of Pseudomonas aeruginosa isolates producing KPC and VIM carbapenemases in diverse genetic clones at tertiary-care hospitals in Medellín, Colombia. Journal of clinical microbiology. 2014;52(11):3978-86. https://doi.org/10.1128/jcm.01879-14
20. Solgi H, Nematzadeh S, Giske CG, Badmasti F, Westerlund F, Lin Y-L, et al. Molecular epidemiology of OXA-48 and NDM-1 producing enterobacterales species at a University Hospital in Tehran, Iran, between 2015 and 2016. Frontiers in Microbiology. 2020;11:936. https://doi.org/10.3389/fmicb.2020.00936