Microbes and Infectious Diseases 2025; 6(2): 701-709

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Identification of multidrug-resistant *Enterobacteriaceae* harboring *bla*$_{\text{NDM-1}}$ gene isolated from surgical site infection at Benha University Hospital

Emira E. Ramadan[1*], Mohamed E. Ramadan[2], Nora M. Nassar[3], Nashwa E. Ahmed[4], Riham N. Ragab[1].

1- Department of Medical Microbiology and Immunology, Faculty of Medicine, Benha University,Egypt,

2- Department of general surgery, Faculty of Medicine, Benha University, Egypt

3- Department of clinical and chemical pathology, Faculty of Medicine, Benha University, Egypt

4-Department of medical biochemistry and molecular biology, Faculty of Medicine, Benha University, Egypt

ARTICLE INFO

Article history:
Received 1 December 2024
Received in revised form 26 December 2024
Accepted 29 December 2024

Keywords:
SSI
MDR
Metallo-beta-lactamase
bla$_{\text{NDM-1}}$.

ABSTRACT

Background: Surgical site infection (SSI) is a major source of morbidity and mortality. It happens within 30 days of a surgical intervention around or at the incision site. Deeper underlying tissues may be involved. In recent years, multidrug-resistant (MDR) organisms are responsible for a growing proportion of illnesses, which present a significant therapeutic challenge. **Aim:** This study aimed to evaluate the antibiotic susceptibility pattern of Enterobacteriaceae isolated from SSI in Benha University Hospital and detect the blaNDM-1 gene in carbapenem resistant isolates. **Methods:** A total of 130 wound samples were cultured on blood agar and MacConkey agar media. Strains were identified by standard bacteriological methods and antibiotic sensitivity testing by VITEK 2 compact system, Detection of MBL (metallo-beta-lactamase) producing isolates phenotypically by double disk synergy test (DDST) and detection of blaNDM-1gene in the resistant strains by Conventional PCR. **Results:** of 120 culture positive wound samples, 53 strains were Enterobacteriaceae (22Klebsiella species, 20 E.coli, 8 Proteus spp and 3 Citrobacter spp), 59% and 30% of Klebsiella spp and E-coli strains respectively were carbapenem resistant, while none of Proteus or Citrobacter spp were carbapenem resistant. Regarding blaNDM-1 detection it was 61.54% and 66.67% for Klebsiella spp and E.coli respectively. **Conclusion:** NDM-1 gene propagation in Enterobacteriaceae is a worrying threat which necessitates several control measures.

Introduction

One of the most frequent sources of infections connected to healthcare is surgical site infections (SSI) [1]. SSIs are responsible for extended periods of hospitalization, persistent incapacity and extra financial cost. They diminish the potential benefits of surgical procedures [2].

SSI is responsible for 38% of deaths in patients in developing countries [3]. Such infections are commonly caused by microorganisms on the patient's skin at the time of surgical incision. The contamination causing SSI depends on the bacterial count, virulence and medication resistance [4].

The commonest microorganisms linked to infections caused by SSIs are *Staphylococcus*

DOI: 10.21608/MID.2024.340858.2376

* *Corresponding author:* amira essam ramadan

E-mail address: *amera.ramadan@fmed.bu.edu.eg*

aureus, Escherichia coli, Klebsiella spp., Proteus spp., Citrobacter spp., Acinetobacter spp., coagulase negative staphylococci and *Pseudomonas aeruginosa* [5].

During the past few years, Multidrug-resistant (MDR) organisms have become a source of a growing number of illnesses, posing a big therapeutic challenge [6] as multidrug resistance implies resistance to three or more antibiotics from variant categories [7]. Among the most significant emerging resistance features is carbapenem-hydrolysing beta-lactamases production, which grant resistance to almost all beta-lactams [8].

New Delhi metallo-beta-lactamase (NDM) is among the most significant enzymes belonging to this category [9]. NDM-1 was identified in bacterial chromosome and plasmid. Plasmids harbouring the blaNDM-1 gene also found to harbour other genes that grant resistance to other groups of antimicrobials such as aminoglycosides, macrolides and sulfamethoxazole. Thus, strains harbouring NDM are resistant to nearly most available drugs. Furthermore, their encoding genes are easily transferred through plasmids and transposons among *Enterobacteriaceae* [10].

In spite of prophylactic antimicrobials usage both pre- and post-operatively as well as other preventive measures, SSIs remain a serious problem, this is mostly linked to the increasing incidence of antibiotic resistance due to misuse of antibiotics which favours emergence of drug resistant bacteria due to selection pressure [11].

This study aimed to evaluate the antibiotic susceptibility pattern of *Enterobacteriaceae* isolated from SSI in Benha University Hospital and detect the $bla_{\text{NDM-1}}$ gene in carbapenem resistant isolates.

Methods

Study Design

A cross-sectional study was done among patients suffering from SSI in Benha University Hospitals and Microbiology and Immunology Department, Faculty of Medicine, Benha University, Egypt from February 2024 to July 2024. The study was carried out in accordance with the ethical standards of the Declaration of Helsinki and has been accepted by the Research Ethics Committee of Benha Faculty of Medicine (RC 40-1-2024). A written consent in Arabic was obtained from all participants before being enrolled in the study.130 wound samples were collected using standard microbiological sample collection methods, 84 by sterile cotton swabs and 46 aspirated pus. Full bacteriological analysis of samples including microscopic examination after Gram staining, culture characteristics and standard biochemical reactions was done [12].

Sample size calculation

All samples were examined by standard bacteriological methods. Out of 130 samples, 53 *Enterobacteriaceae* were detected.

Sample size was calculated as follows:

$$\text{Sample size (n)} = 130 = \frac{Z^2 * (P) * (1-P)}{C^2}$$

Population = 53

Sample size needed = 48 and it was raised to 53

Antimicrobial susceptibility: was done by VITEK system, Biomerieux according to manufacturer's instructions [13].

Phenotypic Detection of metallo-beta - lactamase production: The bacteria were plated on Mueller Hinton agar as recommended by CLSI (2018). Two disks Imipenem (10 ug) and Imipenem / EDTA (10 ug + 750 ug) were then put at a distance of 25 mm. The inhibition zones of both disks were recorded and compared after 16 to 18 hrs of incubations at 35^0C. A zone diameter difference of Imipenem and Imipenem / EDTA disks ≥ 5mm was regarded as positive metallo-B-lactamase.

Polymerase Chain Reaction: DNA was extracted by Quick-DNA™ Miniprep plus Kit, Zymo Research, USA, according to manufacturer's instructions. Detection of blaNDM-1gene in resistant strains by conventional PCR (Master mix Dream Taq.fermentas, life science, Thermo Fisher Scientific) utilizing the following primers: NDM-1 forward primer (5'- GAC CGC CCA GAT CCT CAA -3') and NDM-1reverse primer (5'- CGC GAC CGG CAG GTT -3') [14].

Reaction components were added as follow, Green Master Mix: 12.5 µl, Forward Primer: 1µl, Reverse Primer: 1µl, Template DNA: 5 µl and RNase-free water with a total volume of 12.5µl. After mixing, the reaction mix was loaded into the PCR cycler. Thermal denaturation was achieved by heating the products for 1 minute at 95°C, and primer annealing was made for 30 seconds at 55 °C, 1 minute of extension at 72°C and a final extension at 72°C for 5 minutes. The PCR products were

electrophoresed on an agarose gel (Hopkins and Williams, England) and visualised using a UV trans-illuminator (Biometra, Germany).

Statistical analysis

Study data of demographic traits and clinical criteria were compiled as percentages and frequencies. For continuous data such as age and surgery duration, mean ± standard deviation (SD) was used. The descriptive statistics were enhanced by the addition of visualizations such as bar charts.

Results

Out of 130 patients with SSI included in the study, 76 (58.46%) were males while 54(41.54%) were females and they ranged in ages from 19-65 years old with Mean age (41.96±14.2) years. Different patient characteristics and risk factors were summarized in (table 1)

Positive bacterial cultures were detected in 120 wound discharge samples out of 130 (92.3%). Out of 120 positive samples, 61(50.83%) Gram negative bacilli while 59(49.17) cultures were Gram positive (Figure 1, 2 and 3).

After further identification procedures, 53 *Enterobacteriaceae* strains were identified as follows: 22 *Klebsiella spp*, 20 *E. coli*, 8 *Proteus spp and* 3 *Citrobacter spp.* 8 non *Enterobacteriaceae* gram negative bacilli were also isolated like *Pseudomonas spp.*

Regarding antibiotic resistance pattern of isolated *Enterobacteriaceae*, 30 strains were MDR and were distributed as follow: 16(53.3%) *Klebsiella spp*, 12 (40%) *E.coli*, 2 (6.7%) *Proteus spp* while none of *Citrobacter spp* were MDR. 19 out of 30 MDR strains were carbapenem resistant as shown in (Table 1)

Phenotypic detection of metallo-beta lactamase by double synergy test: 7 of 13 carbapenem resistant *Klebsiella spp* were positive (53.8%), while 3 of 6 carbapenem resistant *E.coli* were positive (50%) (Figure 4).

Detection of blaNDM-1 gene by PCR: Among 19 CRE, the gene was detected in 12 strains (63.2%) as follow: 8 of 13 carbapenem resistant *Klebsiella spp* (61.5%) and 4 of 6 carbapenem resistant *E.coli* (66.67 %) (Figure 5).

Table 1. Characteristics of the patients.

Characteristics of Patients (n=130)		**number**	**Percentage**	**Mean ± Std**
Sex	Male	76	58.46	-
	Female	54	41.54	-
Age(years)	19-65	-	-	41.96±14.2
Surgery duration	> 3 Hours	115	88.46	
	≤ 3 Hours	15	11.54	
Ward	Surgical	85	65.38	-
	Obs/Gyn	45	34.62	-
Diabetes mellitus		13	10	-
Type of wound	Clean	70	53.85	-
	Contaminated	10	7.69	-
	Dirty	30	23.08	-
	Clean contaminated	20	15.38	-
Type of surgery	Emergency	90	69.23	-
	Elective	40	30.77	-

Table 2. Number and percentages of multidrug resistant and carbapenem resistant *Enterobacteriaceae* isolated from surgical site infection (detected by VTEK system).

Enterobacteriaceae	Total Number of isolates (%)(n=53)	MDR isolates (n=30) (56.6% of total)	carbapenem resistant isolates (n=19) (35.8% of total)
Klebsiella spp	22(41.5%)	16(53.3%)	13(68.4)
E. coli	20(37.7%)	12(40%)	6(31.6)
Proteus spp	8(15.1%)	2(6.7%)	0(0%)
Citrobacter spp	3(5.7%)	0(0%)	0(0%)

Figure 1. Distribution of positive and negative culture wound samples among SSI cases.

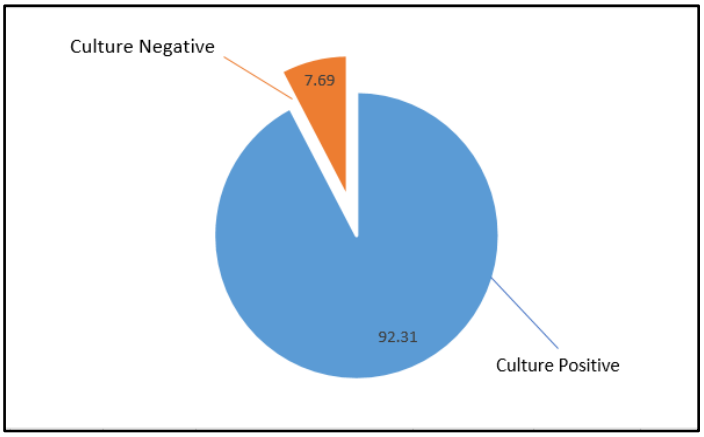


Figure 2. Distribution of Gram positive and Gram-negative organisms isolated from culture positive SSI cases.

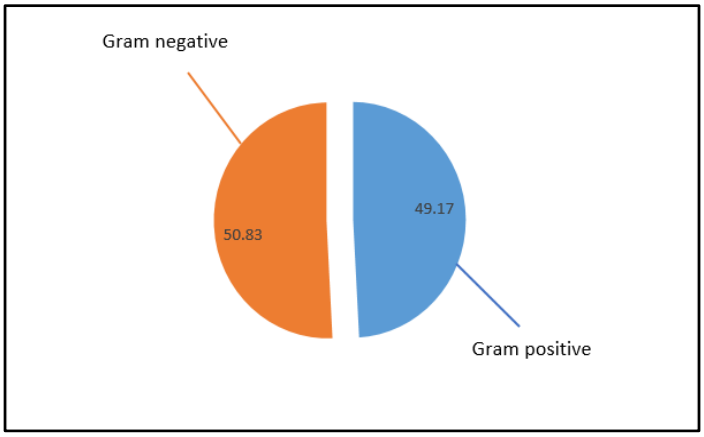


Figure 3. Distribution of *Enterobacteriaceae* among gram-negative organisms.

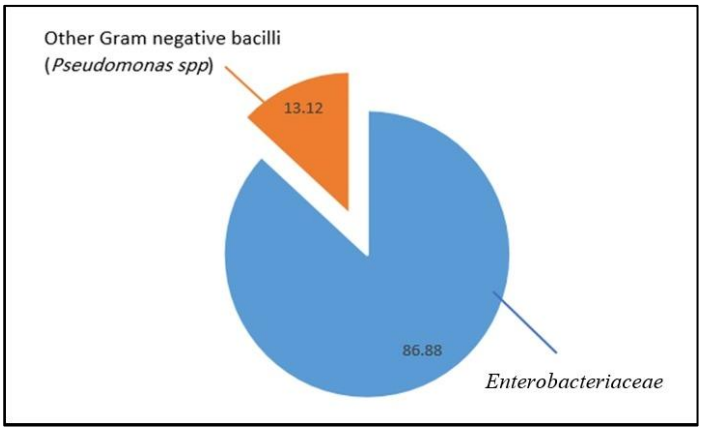


Figure 4. Double disk synergy test (positive test)

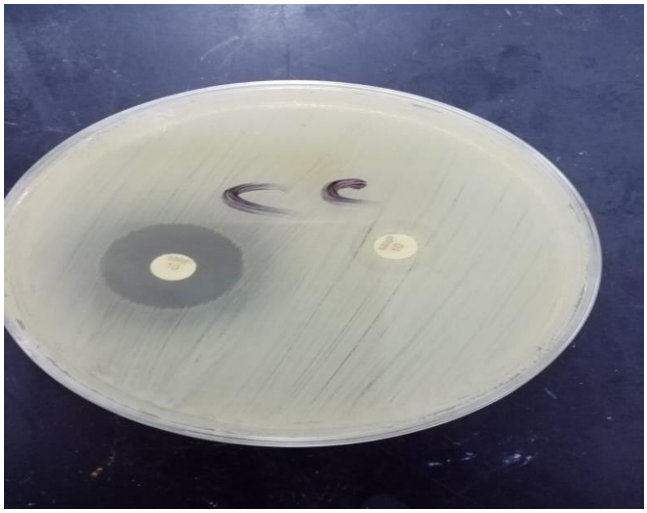
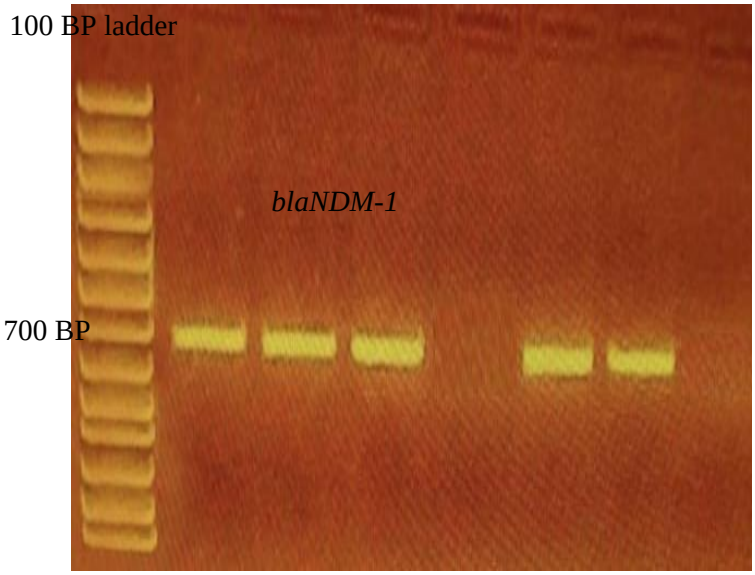


Figure 5. Agarose gel showing the PCR-amplified product of the *bla*$_{NDM-1}$ gene (100 BP ladder).



Discussion

Surgical site infections (SSIs) are among the commonest types of healthcare-associated infections (HAI) and they are increasingly being caused by multi-drug resistant organisms (MDR-Os)[6].

Emergence of NDM-producing *Enterobacteriaceae* became a major worry globally and a public health concern due to few treatment options [15].

In the present study, 120 out of 130 wound samples yielded bacterial growth (92.31 %), this finding agrees with Tilahun [16] who stated that the magnitude of bacterial growth was 95.3% and Roy et al [17] who reported 92.3%. Our finding was different from Gupta [18] et al who reported 100% of wound samples yielded bacteria growth and Misha et al [19] who reported the culture positive rate was 71.7%, it is likely that varying sampling methods and microbial counts could account for variations in culture-positive rates.

In the current study, 61(50.83%) of the isolates were Gram negative, this agrees with Upula et al [20] and Alelign et al [21] who reported that percentage of Gram negative organisms following SSI was 51.2%, while relatively higher percentage were found by Gebissa et al [22] 70.1% and Deepjyoti et al[23] (60.3%). Such variation might be ascribed to variations in the research population, bacterial aetiology and infection prevention procedures in various health-care facilities.

Gram negative bacilli predominance might be due to their multidrug resistance patterns, varied habitats including hospital surfaces and potential contamination from the alimentary tract during surgery [24].

According to this research, the most common isolated species among *Enterobacteriaceae* were *Klebsiella spp* (41.5%).The finding is in line with a study by Kalpana et al [6] with a similar isolation rate (39.8%) as well as according to a study done in Uganda, *Klebsiella pneumonia* was the most prevalent (50%) [25]. However a study done in Addis Ababa, Ethiopia reported that the most prevalent species was *Staphylococcus aureus* [26]. Variations in common hospital-acquired organisms and regional differences in infection prevention practices may be the cause of this discrepancy in the distribution of bacterial species.

The current study detected the rate of MDR *Enterobacteriaceae* isolated from SSI cases to be 56.6% which aligns with a previous study (63%) [6] Meanwhile this rate is higher than a previous study carried out in Egypt (37.2%) [27] And lower than that reported from Hawassa, Ethiopia (93.2%). A key element in the development and dissemination of varying rates of resistance is the empirical treatment strategies, the frequent and careless use of antibiotics by unskilled professionals and the absence of established protocols for antimicrobial use all over the health care settings [28].

In this study, carbapenem resistant *Enterobacteriaceae* prevalence was 35.8% similar to Worku et al [29] who reported that Ertapenem resistance among *Enterobacteriaceae* was 32.9 %. Pavlović et al [30] detected a higher rate of resistance for Imipenem (68.2%) among patients in Surgical Intensive Care Units. Varying permeabilities to distinct carbapenems due to changes in porins may be the cause of varied resistance to these antibiotics [31].

Carbapenem resistance among *Klebsiella spp* was 68.4% that coincides with Raouf et al [32] (69.2%).

Regarding blaNDM-1, 8 of 13 carbapenem resistant *Klebsiella spp* (61.5%) and 4 of 6 carbapenem resistant *E.coli* (66.67 %) tested positive which is close to a previous study of Alruways [33] who reported that blaNDM-1 among carbapenem resistant gram negative isolates was 54%, while another study in Egypt declared that blaNDM gene was 88.7% among CRE [34].

The presence of blaNDM-1 gene in another Egyptian study was higher among MDR *Klebsiella pneumomiae* (78.79 %) and nearly similar among MDR *E.coli* (58.33 %) [8]

A number of reasons including inadequate infection control procedures and antibiotic abuse seems to contribute to the blaNDM-1 gene higher prevalence [35].

However, 36.8% of the CRE isolates in our study tested negative for blaNDM-1 gene raising the possibility of presence of other β-lactamases and carbapenemases genes other than blaNDM-1 and might be also due to presence of novel genes or permeability issues [36].

Conclusion

Multidrug resistance especially carbapenem resistance is an increasing problem which necessitates strict infection prevention strategies and rational use of antibiotics. Further studies are recommended to study the role of other β-lactamases and carbapenemases genes rather than blaNDM-1 in SSIs.

Conflict of interest

The authors declare no conflict of interest

Funding: no funding

References

1- Kaur K, Oberoi L, Devi P. Bacteriological profile of surgical site infections. International Archives of Integrated Medicine 2017; 4, 12.

2- Piednoir E, Robert-Yap J, Baillet P, Lermite E, Christou N. The Socioeconomic Impact of Surgical Site Infections. Frontiers in Public Health 2021; 9: 712461.

3- Monahan M, Jowett S, Pinkney T, Brocklehurst P, Morton DG, Abdali Z. Surgical site infection and costs in low- and middle-income countries: A systematic review of the economic burden. PLOS ONE 2020;15(6): e0232960.

4- Shakir A, Abate D, Tebeje F, Weledegebreal F. Magnitude of Surgical Site Infections,

Bacterial Etiologies, Associated Factors and Antimicrobial Susceptibility Patterns of Isolates Among Post-Operative Patients in Harari Region Public Hospitals, Harar, Eastern Ethiopia. Infection and Drug Resistance Journal 2021; 14: 4629-4639.

5- Moradali MF, Ghods S, Rehm BHA. Pseudomonas aeruginosa lifestyle: a paradigm for adaptation, survival and persistence. Front Cell Infect Microbiol 2017 ;7:39

6- Kalpana A, Gupta N, Jadhav S. Antibiotic resistance pattern of Enterobacteriaceae isolates from Surgical Site Infections with special reference to Multidrug Resistance strains.Teikyo Medical Journal 2023;46:02.

7- Awadallah M, Amer G, Emam S, Ramadan A. Multidrug Efflux Pump In Relation To Antibiotic Resistance Pattern in Escherichia Coli Strains Isolated From Benha University Hospital. Egyptian Journal of Medical Microbiology 2020; 29;2; 87:94

8- Eid R, Ismail G, Yousef L, Hefny H. Phenotypic Identification and Molecular Detection of bla (NDM-1) Gene in Multidrug Resistant Gram Negative Bacilli in Sohag University Hospital. Sohag Medical Journal 2022; 26(2).

9- Hosseinzadeh Z, Ebrahim-Saraie H, Sarvari J, Mardaneh J, Dehghani B, Rokni-Hosseini S, Motamedifar M. Emerge of blaNDM-1 and blaOXA-48-like harboring carbapenem-resistant Klebsiella pneumoniae isolates from hospitalized patients in south western Iran. Journal of the Chinese Medical Association 2018;81; 536:540

10- Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. Clin Microbiol Infect 2014; 20(9):821–830. doi:10.1111/1469-0691.12719

11- Hope D, Ampaire L, Oyet C, Muwanguzi E, Twizerimana H, Apecu RO. Antimicrobial resistance in pathogenic aerobic bacteria causing surgical site infections in Mbarara regional referral hospital, Southwestern Uganda. Sci Rep 2019;9(1):1–1.

12- Cheesbrough M. District Laboratory Practice in Tropical Countries; 2018, Part 2

13- Bratu S, Landman D, Haag R. Rapid spread of carbapenem resistant Klebsiella pneumoniae in New York City: a new threat to our antibiotic armamentarium. Arch. Inter. Med 2005; 165(12): 1430-1435.

14- Salloum T, Arabaghian H, Alousi S, Abboud E, Tokajian S. Genome sequencing and comparative analysis of an NDM-1-producing Klebsiella pneumoniae ST15 isolated from a refugee patient. Pathogens and Global Health 2017 ; 111(4) :166-175

15- Ahmad N, Khalid S, Ali S, Khan A. Occurrence of blaNDM Variants Among Enterobacteriaceae From a Neonatal Intensive Care Unit in a Northern India Hospital. Front Microbiol 2018; 9: 407

16- Tilahun M. Multi-Drug Resistance Profile. Prevalence of Extended-Spectrum Beta-Lactamase and Carbapenemase-Producing Gram Negative Bacilli Among Admitted Patients After Surgery with Suspected of Surgical Site Nosocomial Infection North East Ethiopia. infection and Drug Resistance 2022; 15: 3949–3965.

17- Roy S, Ahmed MU, Uddin BMM. Evaluation of antibiotic susceptibility in wound infections: a pilot study from Bangladesh. F1000 Research 2017; 6:2103.

18- Gupta V, Kaur p, Chhina D, Singh J, Gupta R. Drug-Resistant Strains in Surgical Site Infections after Abdominal Surgery: A

Prospective Study. Journal of Gastrointestinal Infections 2022; 12(02): 106-111

19- Misha G, Chelkeba L , Melaku T. Bacterial profile and antimicrobial susceptibility patterns of isolates among patients diagnosed with surgical site infection at a tertiary teaching hospital in Ethiopia: a prospective cohort study. Annals of Clinical Microbiology and Antimicrobials 2021; 20- 33

20- Upula E, Ekong U, Ekpiken E, Enya J, EzeIje U , Sam-Uket N. Characterization of Virulent Bacterial Isolates Associated with Multi-Drug Resistance among Patients with Surgical Site Infections in Selected Specialist Hospitals in Calabar, Nigeria. Ann. Res. Rev. Biol 2022; 37(12) 75-85.

21- Alelign D, TenaT, Tadesse D, Tessema M, Seid M, Oumer Y, Aklilu A, Beyene K, Bekele A, Abebe G, Alemu M. Bacteriological Profiles, Antimicrobial Susceptibility Patterns, and Associated Factors in Patients Undergoing Orthopedic Surgery with Suspicion of Surgical Site Infection at Arba Minch General Hospital in Southern Ethiopia. Infection and Drug Resistance 2022; 15;2427-2443

22- Gebissa T, BudeB, Yasir M, Mekit S , Noorulla K M . Bacterial isolates and their antibiotic sensitivity pattern of surgical site infections among the surgical ward patients of Asella Referral and Teaching Hospital. Future Journal of Pharmaceutical Sciences 2021 ;7(100)

23- Deepjyoti K, Chandra M, Sumi D, Sarma, Kumar R, Sangeeta DM. Drug resistance profile in postbiliary and gastrointestinal surgical-site infection cases from a tertiary care hospital. Indian Journal of Pharmacology 2023; 55(6):p 399-404.

24- Mythri BA , Kumar SM, Patil AB, Pramod G. Uppar A. Astudy of aerobic bacteriological profile of surgical site infections in a tertiary care hospital. IP Int J Med Microbiol Trop Dis 2020;6(1):42-47

25- Lubega A, Joel B, Lucy NJ. Incidence and etiology of surgical site infections among emergency postoperative patients in mbarara regional referral hospital, South Western Uganda. Surg Res Pract 2017;1-6.

26- Asres GS, Legese MH, Woldearegay GM. Prevalence of multidrug resistant bacteria in postoperative wound infections at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. Arch Med 2017;9 (4):12.

27- Zahran WA, Zein-Eldeen AA, Hamam SS, Sabal MSE. Surgical site infections: problem of multidrug-resistant bacteria. Menoufia Med J 2017;30 (4):1005–1013.

28- Dessalegn L, Shimelis T, Tadesse E, Gebre-selassie S. Aerobic bacterial isolates from post-surgical wound and their antimicrobial susceptibility pattern: a hospital based cross-sectional study. J Med Res 2014; 3(2):18–23.

29- Worku S, Abebe T, Alemu A, Seyoum B, Swedberg G, Abdissa A, et al. Bacterial profile of surgical site infection and antimicrobial resistance patterns in Ethiopia: a multicentre prospective cross-sectional study Annals of Clinical Microbiology and Antimicrobials 2023; 22(96).

30- Pavlović D, Pospišil M, Nađ M, Mijatović V, Luxner J, Zarfel G, et al. Multidrug-Resistant Bacteria in Surgical Intensive Care Units: Antibiotic Susceptibility and β-Lactamase Characterization. Pathogens 2024, 13(5): 411

31- Jamal WY, Albert MJ, Rotimi VO. High Prevalence of New Delhi Metallo-β-Lactamase 1 (NDM-1) Producers among Carbapenem-Resistant Enterobacteriaceae in Kuwait. PLoS ONE 2016;11(3): e0152638.

32- Raouf M, Ghazal T, KassemM,Agamya A, Amer A. Surveillance of surgical-site infections and antimicrobial resistance patterns in a tertiary hospital in Alexandria, Egypt. J Infect Dev Ctries 2020; 14(3):277-283.
33- Alruways M W. Phenotypic and Molecular Characterization of Carbapenemase-Producing Gram-Negative Isolates Detected from Wound Infections. Med forum 2023;43(1)
34- Raheel S A, Mohamed H A, Hessam W F, Abbadi S H, El Sayed A E. Detection of carbapenemase enzymes and genes among carbapenem-resistant Enterobacteriaceae isolates in Suez Canal University Hospitals in Ismailia, Egypt. Microbes and Infectious Diseases 2020;1(1)24-33
35- Garcia-Fernandez A, Miriagou V, Papagiannitsis, Giordano A, Venditti M, Mancini C, et al. An ertapenem-resistant extended-spectrum-β-lactamase-producing Klebsiella pneumoniae clone carries a novel OmpK36 porin variant. Antimicrob Agents Chemother 2010; 54; 4178-418.
36- Baroud M, Dandache I, Araj GF, Wakim R, Kanji S, Kanafani Z. Underlying mechanisms of carbapenem resistance in extended-spectrum β-lactamase-producing Klebsiella pneumoniae and Escherichia coli isolates at a tertiary care centre in Lebanon: role of OXA-48 and NDM-1 carbapenemases. Int J Antimicrob Agents 2013; 41; 75-79.

Ramadan A, Ramadan M, Nassar N, Ahmed N, Ragab R. Identification of multidrug-resistant Enterobacteriaceae harboring the *bla*$_{\text{NDM-1}}$ gene isolated from surgical site infections at Benha University Hospital. Microbes Infect Dis 2025; 6(2): 701-709.