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ORIGINAL RESEARCH

Comparative Study of Surgical Site Infections and Antimicrobial Resistance with Focus on Carbapenem Resistant *Klebsiella pneumoniae*

Renuka Maharjan[1], Bijoy Lakshmi Dewasy[1], Randhir Kumar Singh[1], Reshma Tuladhar[1], Praveer Raj Singh[3], Hanoon P Pokharel[2], Anjana Singh[1]*

[1]Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal
[2]Department of Microbiology, Birat Medical College Teaching Hospital, Biratnagar, Nepal
[3]Kathmandu Model Hospital, Kathmandu, Nepal

***Correspondence author:** Prof. Dr. Anjana Singh, Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal, Email: anjana.singh@cdmi.tu.edu.com

ABSTRACT

Objectives: The aim of this study evaluates the trends in surgical site infections (SSIs), bacterial distribution, antimicrobial resistance, and molecular detection of resistance genes in two different phases.

Methods: The clinical specimens were collected from SSIs patients in both phases. The bacteria were isolated using standard microbiological techniques and further identified by the VITEK 2 system. Phenotypic screening for carbapenemase was conducted through Modified carbapenem inactivation method (mCIM) while presence of bla_{KPC}, *acrA*, and *acrB* genes were detected by PCR.

Results: In phase II, the SSIs rate reduced by 33% particularly due to improvement in prophylactic practices. The number of isolated bacteria decreased by 73.13%, with Gram-negative bacteria remaining predominant. The study reported significant increase in resistance in *Klebsiella pneumoniae* particularly to amikacin (100%), colistin (80%), and tigecycline (20%) whereas found 100% susceptible to doripenem. All carbapenem resistant *K. pneumoniae* were found mCIM positive but bla_{KPC} negative, while *acrA* and *acrB* genes were detected in all the isolates.

Conclusion: Implementation of improved antimicrobial prophylaxis guidelines resulted in a significant reduction in infection rates. However, an increase in antimicrobial resistance among *K. pneumoniae* was observed. Although the *blaKPC* gene was not detected in carbapenem-resistant isolates, the presence of efflux pump genes in all resistant strains suggests their contributory role in resistance. These findings underscore the urgent need for strengthened antibiotic stewardship and continuous surveillance of resistance mechanisms to curb antimicrobial resistance in surgical settings.

Keywords: Antimicrobial resistance, Carbapenem, Comparative, Efflux pump, Surgical site infections

INTRODUCTION

Surgical site infections (SSIs) and antimicrobial resistance (AMR) are the global health crisis which are linked with each other. According to the survey of 2019, AMR has led to deaths of 1.27 million people worldwide (Murray et al., 2022). The emergence of AMR in the surgical settings is more critical as post-operative infections are common complication. The risk of multidrug resistant bacteria in surgeries have increased leading to difficulty in the treatment of infections (Murray et al., 2022). *Escherichia coli* and *Staphylococcus aureus* are the most common bacteria for morbidity in the context of both post-operative infection and AMR (Chaudhary et al., 2017).

According to World Health Organization (WHO), the global incidence rate of SSIs ranges from 3-50% which

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varies according to the type of surgeries (Leaper et al., 2017). The SSIs rate is significantly higher in low and middle income countries (Leaper et al., 2017). Among patients undergoing caesarean sections, the overall infection rate was estimated at 5.6%, with a substantially higher rate of 11.9% reported in African regions. Following the onset of the COVID-19 pandemic, this infection rate has increased steadily (Farid et al., 2023).

In the hospital settings, administration of antibiotics plays a crucial role in preventing infections during surgery (Leaper et al., 2017). Patients with SSIs receive antibiotics seven times higher than other patients (Aiken et al., 2013). During hospitalization, 60% of patients undergoing surgery receive antibiotics and 50% of patients continue taking antibiotics after discharged (Charani et al., 2023). Overuse of antibiotics as prophylaxis and treatment play a significant role in the development of AMR, threatening healthcare system (Nayan et al., 2023). A rise in the AMR has contributed to an increase in healthcare cost and economic burden. Extended spectrum beta-lactamases (ESBLs) and carbapenem resistant Gram negative organisms have concerned the surgeons for the significant impact on SSIs (Gashaw et al., 2018). The lack of effective therapeutic options has made the treatment of infections caused by carbapenem-resistant Gram-negative bacteria-particularly *Enterobacterales*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*-increasingly difficult. As a result, these infections are associated with higher rates of treatment failure, prolonged hospital stays, increased healthcare costs, and elevated morbidity and mortality, thereby posing a serious challenge to clinical management worldwide (Dossim et al., 2019).

Carbapenem resistance is mediated by multiple mechanisms, most notably the production of carbapenemases, including Ambler class A enzymes such as *K. pneumoniae* carbapenemase (KPC), class B metallo-β-lactamases (NDM, VIM, and IMP types), and class D oxacillinases, particularly OXA-48 (Lee et al., 2014; Kanj et al., 2011). Besides, resistance to carbapenem is also mediated by a non-enzymatic method, involving efflux pumps (Dinh et al., 1994). It is necessary to diagnose carbapenem resistant enterobacterales (CRE), particularly carbapenemase producing enterobacterales (CPE) accurately on time to ensure effective treatment for prevention of SSI infections (Cui et al., 2019).

This study analyzed the SSIs rate, negative bacteria, trends in antimicrobial resistance, change in pattern of molecular detection of bla_{KPC} gene and efflux gene in carbapenem resistant *K. pneumoniae* between phase I and phase II studies. The outcome will help the health professionals make decisions regarding selection of antibacterial agent, to reduce antibiotics misuse and emergence of AMR.

METHODS

Study design

The study was conducted in two different time periods: phase I study (October 2021 to October 2022) and phase II study (April 2023 to July 2023) at Birat Medical College Teaching Hospital (BMCTH), Biratnagar. Total SSIs observed was 48.52% in phase I therefore to improve the preoperative prophylaxis antibiotics change was implemented where overall SSIs decreased to 12.27% in phase II.

Isolation and identification of bacterial pathogens

Specimens such as pus, tissue and body fluids were collected from patients showing symptoms of SSIs, under aseptic conditions. Specimens were cultured on MacConkey agar and incubated at 37°C for 18-24h. The identification and antibiotic susceptibility test (AST) were conducted using a VITEK 2 compact system (BioMérieux, USA).

Phenotypic confirmation test for carbapenemase production

The *K. pneumoniae* resistant to at least one carbapenem were selected and further subjected to confirmatory test of carbapenemase production (Pyakure et al., 2021) Confirmation of carbapenemase production was conducted by the Modified Carbapenem Inactivation Method (mCIM) test (Van Der et al., 2015).

Extraction of DNA and detection of bla_{KPC} and efflux pump genes

Bacterial DNA was extracted by the boiling method and extracted DNA was quantitated. Then 25µL of reaction mixture was prepared by mixing 2µL of template DNA, 5µL of 5X Master Mix (FIREPol), 1µL of 10ρmol of each primer (Table 1) and 16µL of ddH_2O nuclease free water. The bla_{KPC} and efflux pump genes (*acrA and acrB*) were detected by amplification using conventional PCR (Omar et al., 2014; Poirel et al., 2011). Primers used for the amplification are summarized in (Table 1).

Table 1: Specific primer sequence used for the amplification of target genes

Gene name	Primers	Primer sequence (5' - 3')	Amplicon size (bp)	Reference
bla_{KPC}	Primer F	CGTCTAGTTCTGCTGTCTTG	798	(Omar et al., 2014)
	Primer R	CTTGTCATCCTTGTTAGGCG		
acrA	Primer F	TGATGCTCTCAGGCAGCTTA	226	(Poirel et al., 2011)
	Primer R	GCCTGGATATCGCTACCTTC		
acrB	Primer F	CGTCTCCATCAGCGACATTAAC	219	Poirel et al., 2011)
	Primer R	GAACCGTATTCCCAACGCGA		

Statistical analysis

The collected data were entered in excel spreadsheet (2011) and subsequently into SPSS for analysis (Version 26.0). Descriptive statistics were calculated to summarize the relevant variables, and the results were presented using text, figures, and tables. Statistical significance difference is considered at $p<0.05$.

Ethics approval and consent to participate

This study was approved by the Nepal Health Research Council (NHRC) (Ref. No. NHRC 234/2020 for phase I and 160/2023 for phase II data collection). Participants were informed about the study's purpose, potential risks, confidentiality of personal information, and their right to voluntarily participate. Written informed consent was obtained from adult participants, while assent was obtained from participants under 18 years of age, along with consent from their parents or legal guardians. Specimens were then collected from all study participants and analyzed according to the study protocol.

RESULTS

In the phase I study, 251 patients were included among which 134(46%) were found culture positive. In phase II study, the infection rate dropped by 33% (Figure 1).

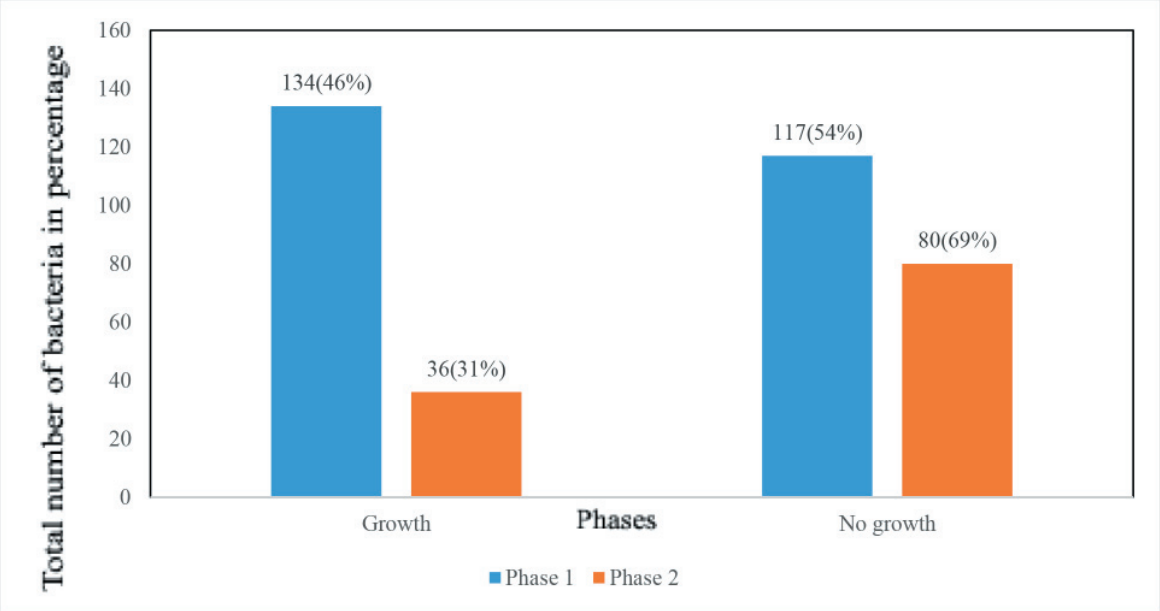

Figure 1: Comparison of prevalence of SSIs in Phases I and II

Distribution of different types of specimen

In phase II only 36 bacteria were isolated. A decrease in 73.13% was observed in phase II compared to phase I. The samples were mainly pus 31(86%), body fluid 4(11%), tissue 1(3%) (Table 2).

Table 2: Distribution of different types of specimen in phases I and II

Specimen	Number of bacteria in phase I N(%)	Number of bacteria in phase II N(%)	Variation rate (%)
Pus	100(75)	31(36)	-69
Body fluid	20(15)	4(11)	-80
Tissue	14(10)	1(3)	-92.85
Total	**134**	**36**	**-73.13**

Prophylactic antibiotics use in two different study period

In phase I, ceftriaxone was the most frequently used antibiotic for preoperative, intraoperative, and postoperative prophylaxis. Other antibiotics administered included cephalexin, azithromycin, ampicillin, clindamycin, amoxicillin–clavulanate, and cefazolin, as illustrated in (Figures 2-4). During this phase, the SSIs rate was 46%. Owing to the high incidence of infections, the findings were communicated to the hospital, and the adoption of an improved antimicrobial prophylaxis regimen was recommended. In phase II, in addition to the antibiotics used in phase I, patients received broader-spectrum agents, including cefixime, piperacillin–tazobactam, ciprofloxacin, amikacin, cefuroxime, and levofloxacin. Following this modification in antibiotic prophylaxis, the SSIs rate decreased by 33%.

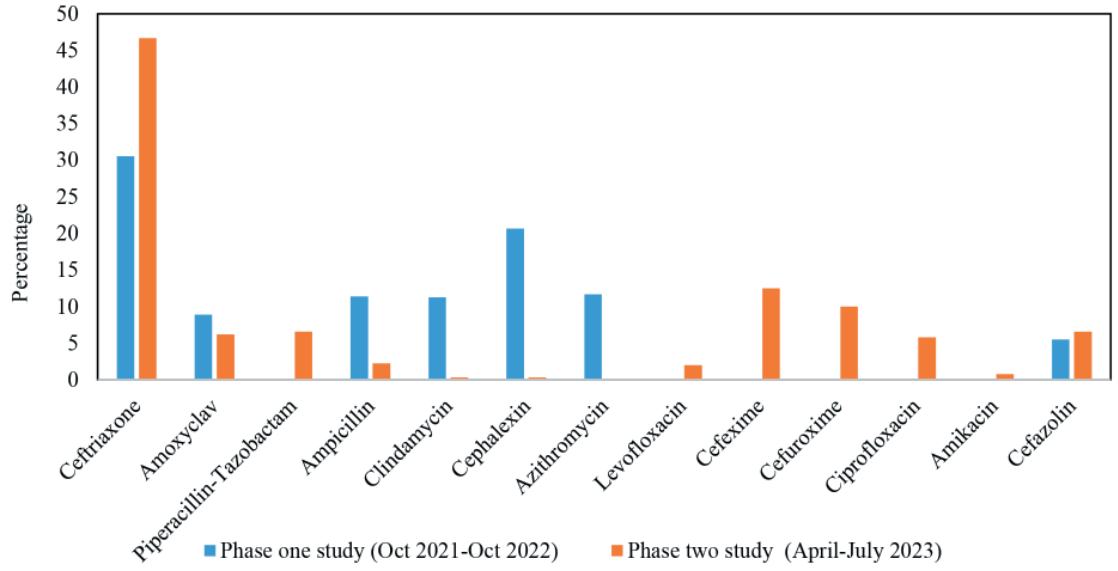

Figure 2: Comparative bar diagram on the pattern of preoperative antimicrobial prophylactic agent used in the patient undergoing surgery

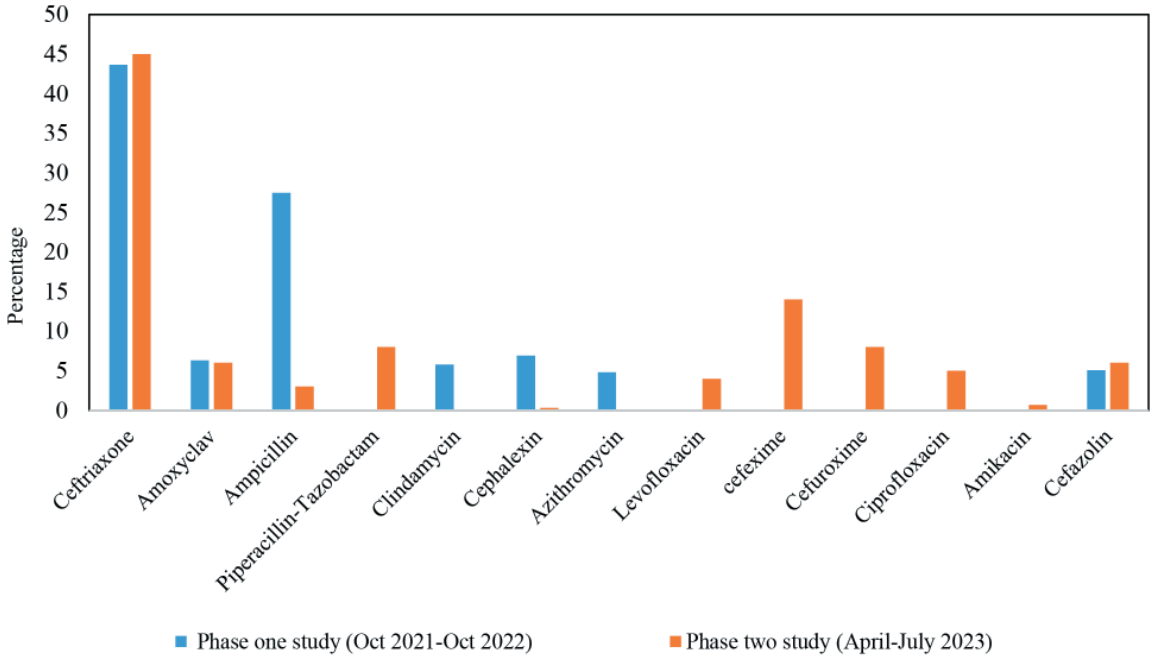

Figure 3: Comparative bar diagram on the pattern of intraoperative antimicrobial prophylactic agent used in the patient undergoing surgery

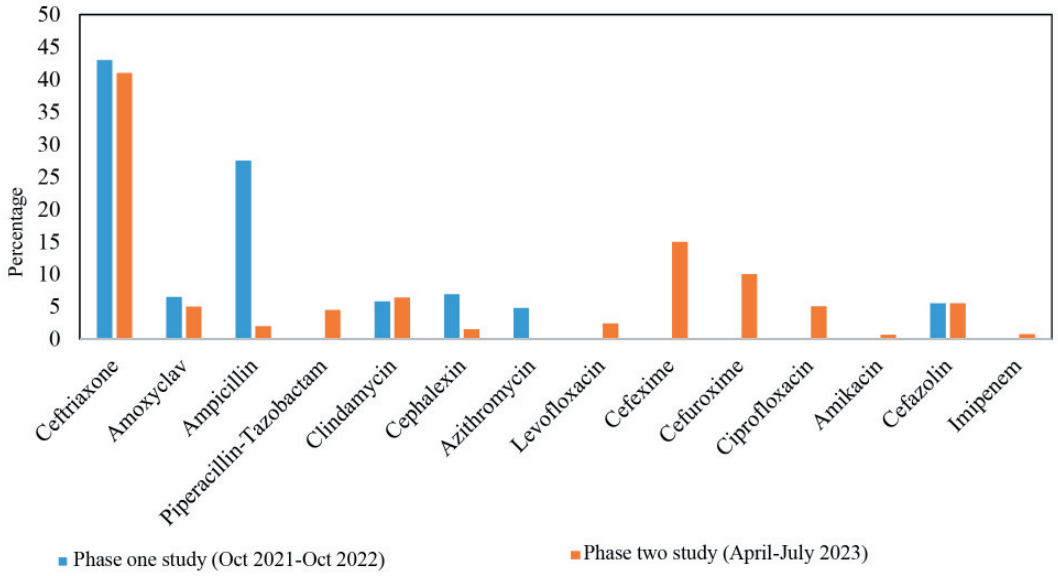

Figure 4: Comparative bar diagram on the pattern of postoperative antimicrobial prophylactic agent used in the patient undergoing surgery

Frequency and distribution of Gram negative bacteria

In phase I, the overall prevalence rate of Gram negative bacteria was 60.44% which included *E. coli* 31(38%), *K. pneumoniae* 24(30%), *P. aeruginosa* 21(26%), *Acinetobacter* spp 4(5%) and *Proteus* spp 1(1%). In phase II, the overall detection rate of Gram negative bacteria was 58.33% accounting *E. coli* 7(33%), *Acinetobacter* spp 4(29%), *K. pneumoniae* 5(24%), and *P. aeruginosa* 5(24%) (Table 3).

Table 3: Comparison of Gram negative bacteria isolated from surgical wounds

Species	Phase I N(%)	Phase II N(%)	Variation rate (%)
E. coli	31(38)	7(33)	-77
K. pneumoniae	24(30)	5(24)	-79
P. aeruginosa	21(26)	5(24)	-76
Acinetobacter spp	4(5)	4(19)	0
Proteus spp	1	0	-100

Antibiotic susceptibility test

In phase II, the resistance rate of *K. pneumoniae* to the majority of the antibiotics had increased significantly compared to phase I study. The resistance rate to amikacin was significantly higher than phase I, with resistance rate of 100% and MIC value of ≥128µg/mL. Compared to phase I, the resistance rate of *K. pneumoniae* to tigecycline and colistin increased from 0% to 20% and 80% respectively. In phase II, doripenem was found 100% sensitive to *K. pneumoniae* (Table 4).

Table 4: Change in antibiotic susceptibility pattern and MIC values

Antibiotics	Phase I		Phase II	
	Resistant (%)	MIC (µg/mL)	Resistant (%)	MIC (µg/mL)
Piperacillin-Tazobactam	45.8	≥128	80	≥128
Ciprofloxacin	45.8	≥2	80	≥4
Gentamicin	29	≥16	80	≥16
Tigecycline	0	-	20	8
Trimethoprime/Sulfamethoxazole	37.5	≥320	80	≥320
Amikacin	37.5	≥64	100	≥128
Cefoperazone/Sulbactam	45.8	≥64	80	≥64
Cefepime	45.8	≥64	80	≥64
Imipenem	45.8	≥16	80	≥16
Meropenem	45.8	≥32	80	≥16
Doripenem	-	-	0	-
Colistin	0	0	80	≥64

Molecular detection of bla_{KPC}, *acrA* and *acrB* genes

During Phase I, a total of 11 carbapenem-resistant *K. pneumoniae* isolates were identified, of which 7 (63.6%) were positive by the modified carbapenem inactivation method (mCIM). In Phase II, the number of carbapenem-resistant *K. pneumoniae* isolates decreased to 4 where all four isolates (100%) were mCIM positive. Detection of the blaKPC gene was not performed in either phase (ND) (Table 5).

Table 5: Phenotypic carbapenemase positive and bla_{KPC} gene in *K. pneumoniae* isolates of phases I and II

Study period	Carbapenem resistant *K. pneumoniae*	mCIM positive *K. pneumoniae*	bla_{KPC}
Phase I	11	7	*ND
Phase II	4	4	*ND

ND: Not detected

All carbapenem resistant isolates were tested for the presence of efflux pump genes (*acrA* and *acrB*). In both phases I and II, efflux pump genes were present in all the carbapenem resistant isolates (Table 6, Figure 5).

Table 6: *acrA* and *acrB* genes in carbapenem resistant *K. pneumoniae* in phases I and II

Study period	Carbapenem resistant *K. pneumoniae*	*acrA*	Percentage (%)	*acrB*	Percentage (%)
Phase I	11	11	100	11	100
Phase II	4	4	100	4	100

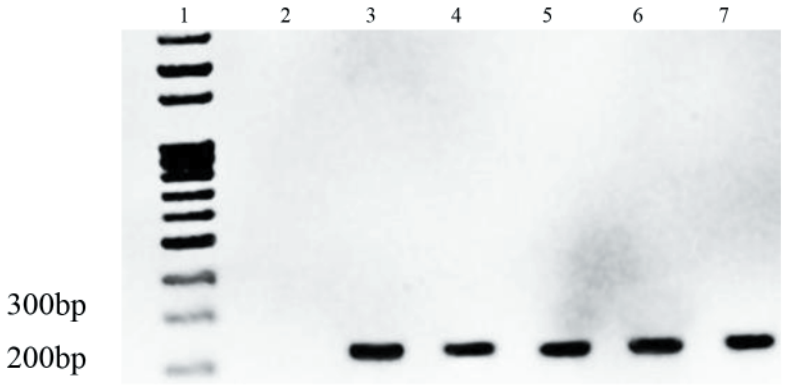

Figure 5: Agarose gel 8% (w/v) image showing efflux pump gene (*acrA* gene-226bp) amplified from carbapenem resistant *K. pneumoniae* isolated from SSIs in phase I. Lane 1: DNA marker, Lane 2: negative control, Lane 3: positive control, Lane 4-7: *acrA* gene of *K. pneumoniae* from SSIs

DISCUSSION

Comparative analysis of SSIs between two study periods revealed that infection rate dropped iin phase II by 33%, highlighting that the prophylactic measures had been effectively implemented and the infection control measures had been enhanced.

In both phases, specimens such as pus, body fluid and tissue were collected from SSIs patients. In phase II study, 36 different bacteria were isolated, which was a significant reduction of 73.13% compared to phase I. In both studies, majority of bacteria were Gram negative bacteria. This finding was consistent with a study conducted in Ethiopia (57.9%) (Razavi et al., 2020; Worku et al., 2023). Furthermore, the prevalence rate of Gram negative bacteria had dropped from 60.44% in phase I to 48.33% in phase II. However there was also a change in the composition of bacteria. In phase I study, *E. coli*, *K. pneumoniae* and *P. aeruginosa* were predominant bacteria. In phase II study, the rate of *E. coli* and *K. pneumoniae* decreased by 77% and 79% respectively.

In this study, the outcome of the changes made in antimicrobial prophylaxis due to high infection rate observed during phase I was analyzed. In phase I, majority of the patients were administered ceftriaxone across all the stages of surgery. Reporting of high infection rate resulted in introduction of more effective antimicrobial prophylaxis. During phase I study, additional broad spectrum antibiotics such as; cefixime, piperacillin-tazobactam, and levofloxacin were introduced, which reduced the infection rate by 33%. A study conducted by Bratzler et al., (2013)

have shown the importance of selecting antimicrobial agents and the time of administration in preventing SSIs. The study suggested cefazolin or cefuroxime for clean surgeries and broader-spectrum agents for surgery with the high risk of contamination (Bratzler et al., 2013). Furthermore, a meta-analysis conducted by Allegranzi et al., (2016) showed that infection rate can be reduced significantly through application of updated version of prophylaxis protocols.

All other bacteria except *K. pneumoniae* were excluded from the study. In phase II, there was a marked increase in antibiotic resistance in *K. pneumoniae* compared to phase I. In phase II study, the resistance to amikacin increased to 100%. An earlier study reported only 23.1% of *K. pneumoniae* were resistant to amikacin (Nepal et al., 2017). The resistant rate to tigecycline had risen to 20%, which is of concern since this antibiotic is often used for the treatment of infection with resistant Gram negative pathogens. Recent study have also reported an increase in resistance to tigecycline, particularly in *K. pneumoniae* (Elgendy et al., 2018). Similarly, a marked increase in colistin resistance was observed between phase I (0%) and phase II (80%). Although colistin is considered a last-line agent for the treatment of multidrug-resistant Gram-negative infections, such a sharp rise in resistance is unexpected and warrants careful interpretation. Several factors may have contributed to this observation. First, the expanded use of broad-spectrum antibiotics in phase II, including agents such as piperacillin–tazobactam, fluoroquinolones, and aminoglycosides, may have exerted indirect selective pressure favouring the emergence or enrichment of intrinsically resistant or heteroresistant *B. fragilis* subpopulations. Second, increased empirical or off-label use of colistin in critically ill patients during Phase II cannot be excluded and may have contributed to resistance selection. Third, anaerobic bacteria such as *B. fragilis* exhibit variable and poorly characterized susceptibility patterns to colistin, and phenotypic resistance may be influenced by methodological limitations of in vitro testing, including inoculum size, growth conditions, and breakpoint interpretation. Importantly, the small sample size in phase II and the absence of molecular characterization of colistin resistance mechanisms (e.g., lipid A modification pathways or *mcr* genes) limit definitive conclusions. Therefore, this finding should be interpreted cautiously and highlights the need for further investigation using standardized susceptibility testing methods and molecular confirmation. Nevertheless, the observed increase raises concern regarding the potential impact of changing antimicrobial practices on resistance patterns and underscores the importance of antimicrobial stewardship. A study conducted in Italy reported 43% of colistin resistance in carbapenem resistant *K. pneumoniae* collected from different hospitals and stated the possibility of evolution of colistin resistance in surroundings with a high prevalence of *K. pneumoniae* producing *K. pneumoniae* carbapenemase (KPC) (Monaco et al., 2014). In phase II study, doripenem was found 100% effective. A dramatic increase in resistance to commonly used antibiotics such as amikacin, tigecycline and colistin emphasizes a critical need of strong antibiotic stewardship initiatives.

Furthermore all the *K. pneumoniae* isolates resistant to at least one type of carbapenem were screened for carbapenemase production phenotypically. In phase II, all the 4 carbapenem resistant isolates were found mCIM positive indicating 100% phenotypic positivity whereas in phase I only 63.6% positivity was obtained. All mCIM positive isolates however were found negative for bla_{KPC} gene, in both phases I and II. The mCIM test is highly sensitive and specific phenotypic method for the detection of carbapenemase production (Li et al., 2019). The 100% mCIM positivity observed in this study is consistent with findings reported by Lee et al., (2019) supporting the high sensitivity of mCIM for the detection of resistant strains.

The isolates exhibiting positive results in the mCIM test being negative for bla_{KPC} gene, highlights that carbapenem resistance in this study was not due to bla_{KPC} gene.

Both *acrA* and *acrB* genes were present in all the carbapenem resistant *K. pneumoniae* in both phases. This findings is similar with a study conducted in Iraq which showed presence of *acrA* and *acrB* genes in all the multidrug resistant *K. pneumoniae* (Abid et al., 2022). The absence of bla_{KPC} gene but presence of efflux genes in all the mCIM positive isolates in this study suggest the common resistance mechanisms, such as efflux pumps to be important contributor for AMR. The lack of the bla_{KPC} gene in all mCIM positive isolates however requires further investigation to delineate other possible mechanisms too. Although bla_{KPC} gene is common genetic element contributing for carbapenem

resistance other mechanisms besides efflux pumps, such as changes in porin channels, and modified beta-lactamases can also be driving factor co-existing to confer resistance. This study involves a comparative analysis of SSIs and AMR during two different study periods to assess the impact of implementing improved antimicrobial prophylactics on SSIs rates and emergence of resistance among bacteria. Phenotyic and genetypic test contributed to a comprehensive investigation of factors contributing to resistance in *K. pneumoniae*. Reduction in infection rates and alteration of resistance profile underscores the importance of antibiotic stewardship strategies. Furthermore, the study focused only on detection of bla_{KPC} and efflux pump genes but other carbapenemase genes such as $bla_{NDM,}$ bla_{OXA-48} and bla_{VIM} were not investigated and porin loss was not considered either. Moreover, various clinical information such as patient mortality, readmission rates, and length of hospital stay were not analysed. Lastly, the short duration of phase II may have limited the observation of seasonal trends or longer-term changes.

CONCLUSION

The study showed a significant reduction in infection rate after implementation of enhanced prophylactic measures. However, increase in the antibiotic resistance, especially in *K. pneumoniae*, highlights the need for continuous surveillance, strong antimicrobial stewardship, and continued exploration of alternative treatment options. Furthermore absence of bla_{KPC} gene and presence of efflux gene in our isolates highlights the role of non carbapenemase based resistance mechanisms, such as efflux pump in carbapenem-resistant *K. pneumoniae* infections.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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