Original Research

Resistance to bacterial infection, complication occurring after cardiac surgery

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Abstract: To analyze the occurrence of resistant bacterial infection in patients undergoing cardiac surgery hospitalized in the surgical specialty hospital, in Erbil city, Iraq. A prospective study was done on a total of 138 patients operated and hospitalized in an intensive care unit and surgical wards. Bacterial isolates identification was done according to cultural characteristics, microscopic examination, some biochemical tests, analytic Profile Index 20E& API *Staph*, confirmed with VITEK® 2 compact system (BioMérieux). Antimicrobial susceptibility for disc diffusion tested to 17 antimicrobial agents. Resistance isolates were confirmed phenotypically for carbapenemase by Rapidec Carba NP Test (bioMe´rieux SA, Marcy-l'E´toile, France) for ESBLs producers by ESBL screening test VITEK 2 system. Molecularly *blaIMP blaTEM, blaKPC, AmpC* and *blaCTX-M* were detected by PCR. In 134 patients, 28.3% of patients got infected post-operatively. The most frequent source of isolation was from ICU patients (75%). Isolated bacteria included gram-positive 29 (54.7%) and gram-negative bacteria 24 (45.3%). Most frequently: *Staphylococcus aureus* (24.4%), each of *pseudomonas aeroginosa*, *Klebsiella pneumonia* (15.1%), *Streptococcus spp.* (11.3%), *Escherichia coli* (9.4%). Whereas included Coagulase Negative Staphylococci species (CoNS) (13.2%) and Enterococci species (5.7) Statistical analysis showed significantly higher sensitive isolates as compared with resistance isolates. Resistance to Carbapenems calss was 18.9% and Cephalosporins class 41.5% of isolates. The antimicrobial resistance pattern indicated that MDR bacterial isolates (81.1%) were widespread. Of the 34 phenotypically ESBL positive isolates, the ESBL genes (*AmpC, blaCTX-M,* and *blaTEM*) were amplified in 7(20.6), 6(17.6) and 6(17.6) isolates respectively. Out of 8 *K. pneumonia* (37.5%) harboring both *blaAmpC* and *bla-CTX-M* genes, while 6(75%) carries *blaTEM*. The *blaCTX-M gene* was found in only 1 (12.5%) out of 8 isolates of *P. aeruginosa*. While *blaAmpC* genotyping revealed that 1(7.7%) out of 13 *Staph. aureus* isolates were harboring it. Finally, 3(60%) out of 5 *E. coli* isolates harboring both *AmpC* and *bla-CTX-M* genes. Cardiac surgery patients wound show increasingly emerging strains of ESBL-producing gram-negative bacteria *K. pneumonia*, *P. aeruginosa* and *E. coli* especially patients prolonged in the intensive care unit.

Key words: Cardiac Surgery; Bacterial Infection; Resistance genes; ICU; MDR.

Introduction

In spite of advances of knowledge in the area of controlling hospital and different systems of scoring tried to predict the infection risk, the surgical site infection (SSI) with antimicrobial resistance clinically important pathogens is increased and remain one of the most common postoperative contraindications and causes remarkable postoperative morbidity and death and could be life-threatening resulting in 100,000 deaths annually in additional health care expenditures. Two to five percent of people undertaking surgery recover from infections at the surgical site with a wide variety of bacteria may be present, either singly or in combination (1).

Over the past decade, the antibiotic resistance of bacteria or multidrug-resistant MDR gram-negative bacilli as extended-spectrum-β-lactamases (ESBL)-producing Enterobacteriaceae and Metallo-β-lactamases (MBLs) have increasing in hospitals and particularly in the intensive care unit (ICU) setting and become a growing problem that is continuing to expand and widely distributed for several gram-negative pathogens and is recognized as a medical issue that raises the morbidity and mortality rates, suggesting duration of hospital stays as well as costs and bad prognosis (2).

Carbapenems are antimicrobials considered the last line of medicines used to treat severe infections (3). The prevalence of Gram-negative bacteria developing broad-spectrum β-lactamase enzymes has resulted in higher use of carbapenem, which has contributed to the broader incidence and dissemination of Enterobacteriaceae generating carbapenemase (4, 5). Until the present time, there is little research on resistance bacteria in an intensive care unit (ICU) and operation ward patients in Erbil hospitals. The aim of this study is the real infections evaluation because of gram-negative bacteria resistance in patients who undergo cardiac operations and admitted to ICU and SW in surgical specialty hospital, in Erbil city, Iraq.

Materials and Methods

Isolation and Identification of bacteria

This study was done from July 2019 to February 2020. It included 138 patients who were admitted surgical specialty hospital, in Erbil city, Iraq. for surgery. The study protocol and the subject were approved by the local ethics committee. It was carried out with patients' verbal and analytical approval before the sample was taken. The specimens collected were wound swabs by dry sterile cotton-tipped swabs or aspirated pus by sterile disposable syringes from the surgical wounds. bacterial

Table 1. Oligonucleotide primer sequences for antibiotic resistance genes.

Target genes	Primer	Sequence(5' 3')	Expected PCR product size (bp)
blaTEM	blaTEM -F	TTGATCGTTGGGAACCGGAG	209
	blaTEM -R	AATAAACCAGCCAGCCGGAA	
AmpC	AmpC-F	AATGGGTTTTCTACGGTCTG	191
	AmpC-R	GGGCAGCAAATGTGGAGCAA	
blaCTX	blaCTX-F	AATCACTGCGTCAGTTCAC	701
	blaCTX-R	TTTATCCCCCACAACCCAG	

Isolation and identification were done regarding standard microbiological techniques, the swab samples were cultured on blood agar (Oxoid Ltd., Basingstoke, UK), MacConkey agar (Oxoid Ltd., Basingstoke, UK), and incubated at 37°C under aerobic conditions for 18–24 hours. The pure colony of each type of bacterial isolates was identified according to cultural characteristics, microscopic examination1, some biochemical tests, analytic Profile Index 20E& API Staph (6). Moreover, the identification of bacterial isolates was confirmed using an automated bacterial identification system VITEK® 2 compact system (BioMérieux).

Antimicrobial susceptibility testing

Fifty-three bacterial isolates were examined for susceptibilities to a total of 17 antimicrobial products, according to the Clinical and Laboratory Standards Institute (7) manual. The bacteria were cultured on Muller-Hinton Agar plate then Ampicillin (AM), Vancomycin (VA), Amoxicillin (AX) Amikacin (AK), Gentamycin(GMN), Imipenem (IPM), Meropenem (MEM), Ceftriaxone (CRO), Ceftazidime(CAZ), Cefotaxime (CTX), Ciprofloxacin (CIP), Clindamycin (DA), Methicillin (ME), Tetracycline (TE), Erythromycin (E), Azithromycin (AZM) and Sulfamethoxazole and trimethoprim (SXT)) disks (Himedia, Mumbai, India) were placed on the media in 20-30 mm with other disks. The plates were incubated for 18-24 hours at 37°C then the results were recorded.

Rapidec Carba NP Test (bioMe´rieux SA, Marcy-l'E´toile, France) manufacturer's instructions regarded the phenotypic detection of carbapenemase-producing bacterial isolates (the resistance of the isolates to imipenem (IPM) or meropenem **(**MEM) or both of them). This test relies on identifying the hydrolysis of the β-lactam ring of carbapenem antibiotics (8). In brief, isolated colony cultures of bacterial isolates were grown onto Mueller-Hinton agar plates at 37°C for 18–24 h. An inoculum corresponding to a one calibrated full loop (10 μl) of colonies of tested strain is taken from a culture plate and mixed into the cell. Visual reading of the plate is made at 37°C after 30 min of incubation, if required the time was prolonged. A positive test result is indicated by a red to yellow or red to orange color changes. ESBLs producers were screened by ESBL screening test by VITEK 2 system utilizes the growth response to ceftazidime, cefepime, and cefotaxime in combination with or without clavulanic acid considered indicative of ESBL production.

Molecular carbapenemase and ESBL screening

For PCR analysis, the genomic DNA of bacterial isolates was prepared using the DNA extraction kit (Promega) according to manufacture instructions. Purified bacterial genomic DNA was transferred into a new tube and stored at −20°C. An aliquot of 2 μl of the supernatant was used as the DNA template for PCR analysis. Resistant gene identification: polymerase (Promega, Madison, Wisconsin, USA) in accordance with the manufacturers' protocols. Carbapenem-resistant isolates were screened by standard PCR for the presence of the following carbapenemase-resistant genes *blaTEM*, *AmpC* and *blaCTX-M* PCR amplification was carried out. The list of oligonucleotide sequences used in this study is shown in Table 1.

Statistical analysis

The statistical analysis of data was done by Graph-Pad Prism 6.

Results and discussion

Out of 138 wound specimens, 28.3% were culture positive aerobically for bacteria Table 2 and Figure 1 present the patients' demographic. The most frequent source of isolation was from ICU patients 27 (75). The Gram-positive bacteria were predominant, 29 (54.7%) in comparison to gram-negative bacteria, 24 (45.3%). The most isolated bacteria included *Staphylococcus aureus* (24.4%), each of *pseudomonas aeroginosa* and *Klebsiella pneumonia* (15.1%), *Streptococcus spp*. (11.3%), *Escherichia coli* (9.4%). Residues were Coagulase Negative Staphylococci species (CoNS) (13.2%) and Enterococci species (5.7) as shown in Table 3. wound infection after cardiac surgery was recorded in 36 (9.4%), 292(4.1%) and 16.4% patients (9-11) Staphylococcus aureus (16%), Staphylococcus epidermidis (12%), Pseudomonas aeruginosa (6,6%), Escherichia coli (5%), was detected from Cardiac Surgery

Table 2. Number and percentage of bacterial infection according to number and type of surgery.

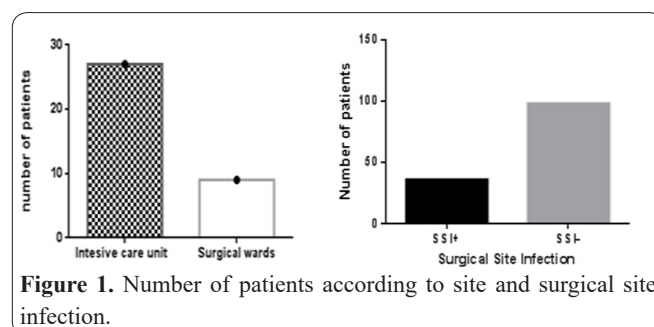| Surgery Type	N (%)	SSI+	SSI-
Emergency Surgerya	97 (70.3)	29 (29.9)	68 (70.1)
Elective Surgery	41 (29.7)	10 (24.4)	31 (75.6)
Total	138	39 (28.3)	99 (71.7)

Figure 1. Number of patients according to site and surgical site infection.

Table 3. The detected pathogenic bacteria from patients

Types of bacteria	N	%	Gram stain %
Staphylococcus aureus	13	24.5	Gram-positive
Coagulase-negative Staphylococci	7	13.2	Gram-positive
Streptococcus spp.	6	11.3	54.7%
Enterococcus spp.	3	5.7	
Escherichia coli	5	9.4	
Pseudomonas aeruginosa	8	15.1	Gram-negative
Klebsiella pneumoniae	8	15.1	
A. baumannii	2	3.8	45.3%
Enterobacter spp.	1	1.9	

patients at surgical Site Infection (12).

All 53 bacterial isolates were subjected to commonly use 17 antibiotics in the study site by disc diffusion method. Statistical analysis showed significantly higher sensitive isolates as compared with resistance isolates (P < 0.05) (Figure 2). Most of the isolates 94.4%, 54.7% were resistant to penicillin class followed by Sulfonamides sulfamethoxazole and trimethoprim 62.3% Macrolides 60.4% and 32.1% Methicillin 33.9% Table 4. The Antimicrobial Resistance pattern showed that the majority of the isolated bacteria were MDR (81.1%)and pandrug-resistant (PDR) (3.8%), whereas extreme drug-resistant (XDR) accounted for 15.1% isolates (Figure 3). Resistance isolates to Carbapenems were 18.9% and to Cephalosporins, the class was 41.5% as in Figure 4.

In this study, the ESBL-producing detected phenotypically in 41.5% of isolates while the percentage resistance to Carbapenems was 18.9% of isolates different proportion was detected in the previous result. Screening Beta-Lactam resistant *k. pneumonia* detected in 58.4% of isolates in Hilla, Iraq (13). Extended-spectrum β-lactamases (ESBLs) producer found in (100%) and (23%) of *E. coli* and *A. baumannii* isolate respectively (14). Important improvements have been observed in P. aeruginosa's tolerance against some β-lactam class antibiotics. Resistance genes in β-lactamase-producing P. aeruginosa have been showing a growing pattern (15). Recently, Iraqi hospitals showed a dramatic variation
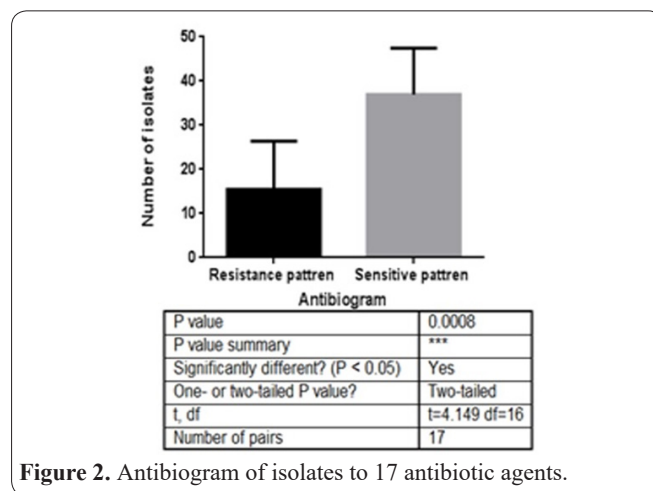

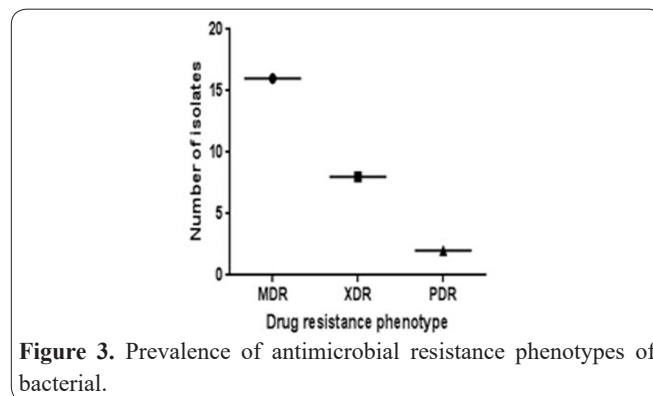**Figure 2.** Antibiogram of isolates to 17 antibiotic agents.

Figure 3. Prevalence of antimicrobial resistance phenotypes of bacterial.

Table 4. The antibiogram of isolated bacteria causing SSI in ten antimicrobial classes.

Antibiotic Classes	Antibiotic name	Resistant isolates, N (%)	Sensitive isolates N (%)
Penicillins	Ampicillin (AM)	50 (94.4)	3 (5.6)
	Amoxicillin (AX)	29 (54.7)	24 (45.3)
Glycopeptide	Vancomycine (VA)	8 (15.1)	45 (84.9)
Aminoglycosides	Amikacin (AK)	12 (22.6)	41 (77.4)
	Gentamycin(GMN)	10 (18.9)	43 (81.1)
Carbapenems	Imipenem (IPM)	4 (7.5)	39 (92.5)
	Meropenem (MEM)	6 (11.3)	47 (88.7)
Cephalosporins	Ceftriaxon(CRO)	8 (15.1)	45 (84.9)
	Ceftazidime(CAZ)	7 (13.2)	46 (86.8)
	Cefotaxime (CTX)	7 (13.2)	46 (86.8)
Quinolones	Ciprofloxacin (CIP)	9 (16.9)	44 (83.1)
Lincomycins	Clindamycin (DA)	11 (20.8)	42 (79.2)
Methicillin (ME)	Methicillin (ME)	18 (33.9)	35 (66.1)
Tetracycline	Tetracycline (TE)	13 (24.5)	40 (75.5)
Macrolides	Erythromycin (E)	17 (32.1)	36 (67.9)
	Azithromycin (AZM)	32 (60.4)	21 (39.6)
Sulfonamides sulfamethoxazole and trimethoprim	Sulfamethoxazole and trimethoprim (SXT)	33 (62.3)	20 (37.7)

Figure 4. The percent of carbapenems and cephalosporins resistance isolates.
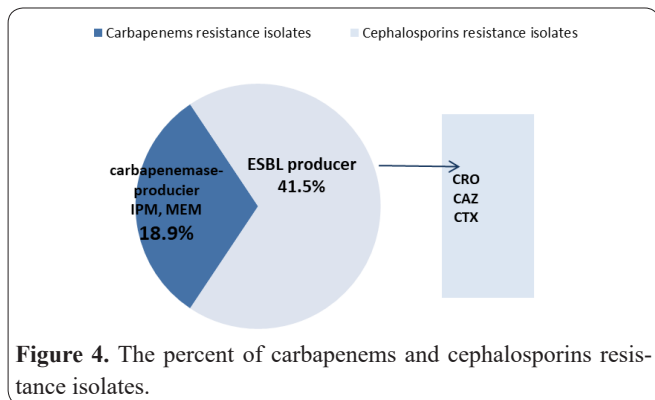
in the resistance pattern of gram-negative to carbapenems this investigated by researchers in a studied hospital in Wasit (16). (34.95%) in Al-Diwaniyah hospitals (17). Another study showed the occurrence of 40% in a studied hospital in Babylon, Iraq (18). Furthermore, (68.34%) in studied hospitals in Karbala province (19) However, earlier studies reported lower prevalence (8%) in studied hospitals of Baghdad (8%) (20) Najaf (12.4%) (21) and Duhok (12.7%) (22).

The present research highlights the frequency of genes that belong to various types of β-lactamase (bla): a broad range of β-lactamase genes. We have previously detected various β-lactamases in our laboratory using phenotypic methods in 53 confirmed bacterial isolates. In the present analysis, these isolates were characterized for their genotypes with respect to the defined genes. Of the 34 phenotypically ESBL positive isolates, the ESBL genes (*AmpC, blaCTX-M,* and *blaTEM*) were amplified in 7(20.6),6(17.6) and 6(17.6) isolates respectively Table 5 and Figure 5. Out of 8 *K. pneumonia* (37.5%) harboring both *blaAmpC* and *bla-CTX-M* genes, while 6(75%) carries *blaTEM* (Figure 6). The *blaCTX-M gene* was found in only 1 (12.5%) out of 8 isolates of *P. aeruginosa* (Figure 7). While *blaAmpC* genotyping revealed that 1(7.7%) out of 13 *Staph. aureus* isolates were harboring it (Figure 8). Finally, 3(60%) out of 5 *E. coli* isolates harboring both *AmpC* and *bla-CTX-M* genes in the
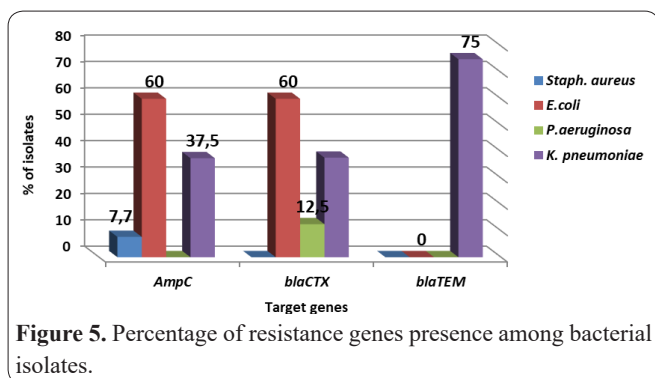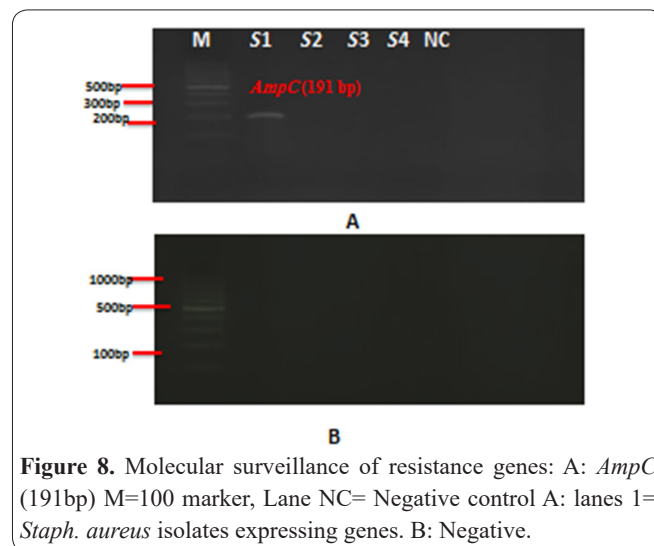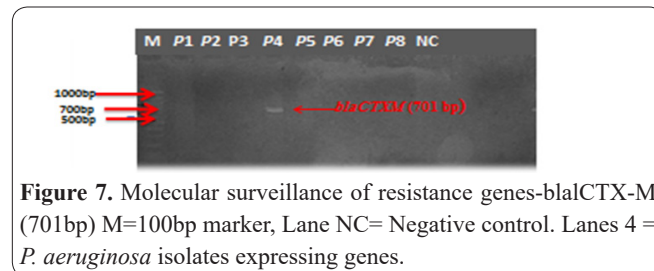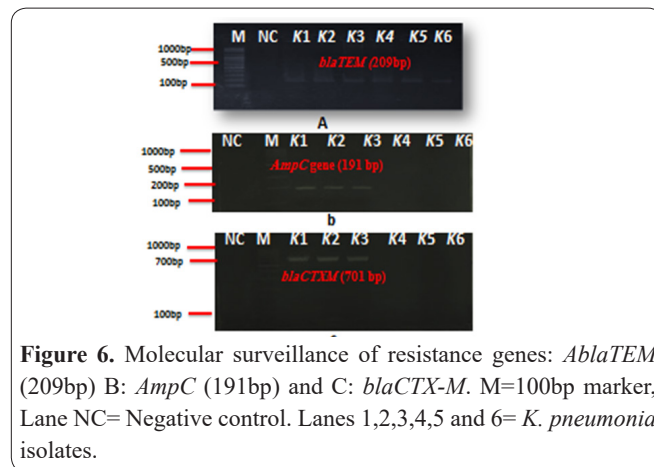

Figure 5. Percentage of resistance genes presence among bacterial isolates.

Figure 6. Molecular surveillance of resistance genes: *AblaTEM* (209bp) B: *AmpC* (191bp) and C: *blaCTX-M*. M=100bp marker, Lane NC= Negative control. Lanes 1,2,3,4,5 and 6= *K. pneumonia* isolates.

Figure 7. Molecular surveillance of resistance genes-blalCTX-M (701bp) M=100bp marker, Lane NC= Negative control. Lanes 4 = *P. aeruginosa* isolates expressing genes.

Figure 8. Molecular surveillance of resistance genes: A: *AmpC* (191bp) M=100 marker, Lane NC= Negative control A: lanes 1= *Staph. aureus* isolates expressing genes. B: Negative.

wound of cardiac surgery infections as in Figure 9. Cardiac surgery patients wound show increasingly emerging strains of ESBL-producing bacteria *K. pneumonia*, *P. aeruginosa*, *E. coli* and *Staph. aureus* especially the patients prolonged in the intensive care unit.

A study in south Taiwan detected 2 isolates among 37 of *K. pneumonia* producing blaKPC gene (23) while other study investigated that all selected *E. coli, Klebsiella spp.* and *P. mirabilis* Isolated from Egyptian Hospital were negative to *AmpC* (24). In a study from Iran,
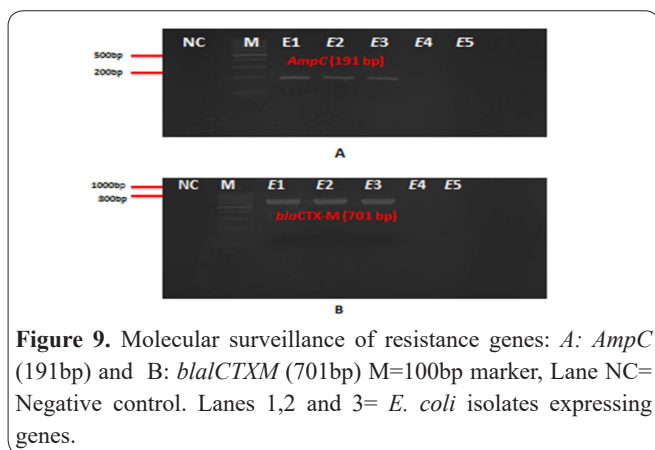
Table 5. Detection of antibiotics resistance genes in bacterial isolates.

Pathogenic bacteria	Total isolates	Isolates carry resistance gene (%)		
		AmpC	*blaCTX-M*	*blaTEM*
Staph. aureus	13	1 (7.7)	0	0
E.coli	5	3 (60)	3 (60)	0
P.aeruginosa	8	0	1 (12.5)	0
K. pneumoniae	8	3 (37.5)	3(37.7)	6 (75)
Total	34	7 (20.6)	6 (17.6)	6 (17.6)

Figure 9. Molecular surveillance of resistance genes: *A: AmpC* (191bp) and B: *blalCTXM* (701bp) M=100bp marker, Lane NC= Negative control. Lanes 1,2 and 3= *E. coli* isolates expressing genes.

the *blaCTX-M* gene was detected only in one isolate (25). The high prevalence of *CTX-M genes* in Enterobacteriaceae leads to the transfer of *bla-CTX-M* genes from the chromosomes to plasmids (26). Due to their broad-spectrum, major guidelines are recommended Carbapenems as empirical treatment for critically ill patients at risk of multidrug-resistant bacteria producing extended-spectrum beta-lactamases (ESBL) with resistance to cephalosporins of the third generation. as therapy of surgical infections. This growing use of carbapenems causes selective pressure on bacteria to develop resistance to carbapenems (27-30).

It can be concluded that infection occurs when the pathogen enters the host body and stimulates the immune system. The effect of this counteraction is often proven to be disruption of the body's normal functioning. It is important to note that if the invader can remain alive and active outside the immune system, the disease becomes acute, but if it enters the system and chooses a host body point to accumulate to resist its destruction. Slowly Chronic disease will be observed (31-38). Therefore, in addition to the susceptibility of the disease in humans and the reduction of the body's general resistance to an infection, the pathogenicity of the invading microbe must be sufficient. To be able to provide a favorable environment in the body for diseases. Our body to defend against microorganisms as causative agents of infection (important infectious agents include viruses, bacteria, rickettsia, fungi and parasites) (39-44).

In conclusion, resistance to bacterial infection Postoperative heart surgery patients at the study site also pose a big concern. Among the bacterial isolates, there was an unprecedented MDR level of 81.1 percent and resistance to the widely used antibiotics. It is highly advised that antibiotic treatment should be driven by trends of antimicrobial susceptibility, regular testing of bacterial resistance infection with and its source of antibiotic susceptibility profile. Finally, to prevent cardiac surgery patients from resistance bacterial infection Preoperative preparations pertaining patients and surgical team with standard sterilization procedures, application of prophylactic drugs and standard and sterilized surgical technologies handling of surgical incisions and wounds are considered and performed one by one.

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