Effect of X-ray radiation on Multi-drug Resistant (MDR) Enterobacteriaceae & P.aeruginosa isolated from burn unit patient. The phenotypic and molecular study, Biofilm formation.

Noor Nadhom khalaf[1], Mohammed Jasim alfeehan[2], Shaymaa Hafez Maiteb[3]

[1]*M.Sc. student \ College of Medicine –University of Anbar*

[2]*FIBMS(plastic and Reconstructive surgery) / University of anbar, college of medicine, department of surgery*

[3]*PhD in Medical Microbiology /University of Anbar, College of Pharmacy*

KEYWORDS

X-ray radiation, OXA-23, OXA-48, bla-TEM, Omp A, Multi-drug resistance (MDR), Burn infection.

ABSTRACT

Background: Multi-drug-resistant (MDR) bacteria are a significant global health concern due to their elevated rates of morbidity and mortality in burn patients. In This study investigated the presence of multidrug-resistant pathogenic bacteria in individuals with burn injuries, the formation of biofilms, and the impact of X-ray radiation.

Method: For this study Clinical swabs about120 burn patients were obtained between December 2023 and June 2024 from burn units at Ramadi and Falluja Teaching Hospitals. Both conventional and Vitek2(bioMérieux) methods were used to identify the bacterial isolates. The isolates were then divided into three multi-drug resistance groups. The Colorimetric method (MTP) detected biofilms. To detect blaOXA-23,blaOXA-48, Omp, and blaTEM resistance genes, multiplex PCR was used. This research used two lumbosacral spine X-ray levels. Two levels of radiation were used 40 (80KV) mAs and 51.2 (85KV).

Result: Results of burn isolation revealed 30(27%) P.aeruginosa, 18(16%) k.pneumoniae, 7(6%) E.coli, 7(6%) P.mirabilis. Of the all isolates, 100% produced biofilm. The Multiplex-PCR analysis revealed that the blaTEM genes were the predominant ones, constituting 30% of the samples. Subsequently, blaOXA-23 constituted 24.5% of the cases, whereas Omp accounted for 22.7% and blaOXA-48 for 13.6%. The radiation impact of X-rays produced by two dosages, 40(80KV)mAs for 125msec and 51,2(85KV) mAs for 160msec, demonstrates that there is no effect or alteration in the resistance capacity of all the bacterial isolates tested.

Conclusion: This study showed that burn unit isolates produce ESBLs, which is a major concern in the intensive care unit due to its high morbidity and potential mortality. These data match antibiotic phenotypic test results showing substantial cefepime resistance. All isolates forming biofilms, this may association with high pathogenicity. This demands quick intervention to manage nosocomial outbreaks. No X-ray effect was seen on susceptibility a burn unit bacterial isolate.

1. Introduction

Antimicrobial agent resistance has become increasingly widespread in recent years and is a significant global public health according the World Health Organisation. The rise of multidrug-resistant microorganisms has been cited as the cause of the rise in morbidity and mortality caused by microbial infections [1]. Microorganisms that are resistant to numerous medications, known as MDR infections, present substantial challenges for burn intensive care units .[2]. Colonisation of burn wounds by opportunistic bacteria such as Pseudomonas aeruginosa, E.coli and Klebsiella

pneumoniae has led to disease outbreaks in burn units worldwide[3].

The empirical antibiotic treatment for multidrug-resistant Pseudomonas aeruginosa has resulted in unfavourable outcomes, including high rates of illness, death, prolonged hospital admissions, and significant financial and therapeutic burdens on both healthcare systems and individuals [4]. K. pneumoniae is also a major nosocomial pathogen that can colonise skin and mucosae and cause serious local and systemic wound infections. Antibiotic resistance, especially to carbapenem, fluoroquinolones, and colistin, is spreading, leaving few treatments for MDR K. pneumoniae infections [5].

Another issue associated with burn infections is the formation of biofilms, which are composed of host proteins and a mucous layer. Biofilms are essential for pathogenicity, as they establish an optimal environment for bacterial growth and the development of resistance to therapy. A higher tolerance and resistance to medications, as well as persistent inflammation, are associated with biofilm production [6] [7]. Many studies have examined Pseudomonas aeruginosa, but little is known about other dangerous bacteria that produce biofilms and their relationship to antibiotic-resistant bacteria in burn patients [8].

Studies conducted have revealed a concerning prevalence of bacterial infections that produce β-lactamase and carbapenemase enzymes, as well as an increasing resistance to other antibiotics. [9]. Carbapenemase enzymes are the most prevalent mechanism by which carbapenem resistance develops. In contrast to Escherichia coli or other Enterobacterial species, Carbapenem resistance has been identified in Enterobacteriaceae, with Klebsiella pneumococcal resistance being the most prevalent. Additionally, Carbapenem resistance has been observed in non-ferm bacteria[10]. Several gene products, including Omp have been identified as critical factors in the promotion of adhesion and the production of biofilms in bacterial pathogen, owing to the intricate character of biofilm development[11]. It has also been extensively recognised that the blaTEM genes are the most prevalent genes that confer antibiotic resistance in pathogenic bacteria on a global scale[12]. The Class D carbapenemase (OXA enzymes) are the primary cause of resistance in A. baumannii globally. Carbapenem-inactivating enzymes are also included[13].

Recently, there has been progress in utilising X-ray radiation to eliminate microorganisms without causing any harm to the patient[14]. This finding implies the possibility of utilising X-ray technology to eradicate microorganisms within living organisms. Bacteria have a highly condensed genome, making them vulnerable to either cell death or genetic changes when exposed to diagnostic levels of X-ray radiation[15].

2. Methodology

Data sources

For the study, a total of 120 clinical samples were obtained from burn patients between December 2023 and June 2024. The samples were obtained from the burn units of Ramadi Teaching Hospital and Falluja Teaching Hospital. The patient's permission to collect a sample was acquired.

Specimens

Swab samples are obtained from various body regions using a sterile transport medium swab. These samples are then cultured on selected media, following bacteriology standards, for a period of 24 hr at a temperature of 37 °C.

Bacterial species identification.

Gram-stained smears were used to examine all samples under a microscope. The specimens were introduced onto appropriate culture media. The presence of microbial growth was identified using established microbiological methods following the suggested duration. A variety of biochemical assays, including the triple sugar iron (TSI) fermentation test, oxidase, indole, motility, urease, and

arginine hydrolysis, were employed to identify the species, as well as the Vitek2 system (bioMérieux).

Antibiotic susceptibility test

The Kirby Bauer's Disc Diffusion method is employed to perform the antibiotic susceptibility test on bacterial isolates. The isolates were grown on Mueller-Hinton Agar at a concentration of 0.5 McFarland for 24 hr at a temperature of 37 °C. [16], [17] A total of 10-14 distinct antibiotics were utilised. Interpretation of results is based on the CLSI-2023 recommendations[18]. Vitek2 system(bioMérieux) is also used for antibiotic susceptibility tests.

Biofilm Formation Quantitative Assay

Biofilm formation is quantified using a colourimetric microtiter technique (Spectrophotometric method)[19]. To determine the quantity of biofilm generated by a microorganism, the Microtiter plate (MTP) assay employs a microplate reader and is a precise quantitative method. A turbidity adjustment of 0.5 McFarland is performed on the bacterial suspension in Brain Heart Infusion Broth (BHI), to which (1%) glucose is added. A concentration of 5.106 cfu/ml is achieved by diluting the bacterial suspension by a factor of 20 (1/20). In order to achieve a final concentration of 5.105 cfu/ml (tenfold dilution 1/10), 180 µl of (BHI) containing (1%) glucose is mixed with 20 µl of bacterial suspensions in a sterile polystyrene microplate with 96 flat-bottomed wells. 24-hour incubation at 37°C is performed on the microplates. Biofilms are generated by the microorganisms that adhere to the walls of microplate wells. With only 100 microliters of Crystal violet, these biofilms were stained for 15 minutes. Phosphate-buffered saline (PBS) at a pH of 7.2 is utilised to rinse the microplate wells twice in order to eliminate any free-floating cells prior to staining.

Following this, the safranin-stained wells of microplates are cleansed twice with Phosphate-buffered saline (PBS) in order to eliminate the stain. After allowing the wells of the microplate to dry, the dye present in the biofilms that covered the walls of the microplate can be dissolved using 150 µl of 100% ethanol. The microplate is quantified at a wavelength of 570 nm, utilising spectrophotometry and a microplate reader. Duplicate investigations are performed for each. Negative controls are blanks, which are inoculated wells containing sterile (BHI) supplemented with (1%) glucose. By utilising the blank absorbance measurements, the presence or absence of biofilm formation in the isolates can be determined (Figure 1). The cut-off value (ODc) can be used to categorise isolates as either biofilm producers or non-producers (Table 1)[19].

Figure (1): Measurement of biofilm formation by MTP method

Table (1): Cut-off value (ODc) and biofilm formation .

Cut-off OD value	Biofilm formation
OD≤ 0.08324	No biofilm
0.15-0.24	Weak biofilm
0.25-0.39	Moderate

0.3	**Strong**

Molecular Analysis

DNA extraction

Following the manufacturer's instructions, the Bosphore extraction versatile Spin Kit (Anatolia genworks-Istanbul, Turkiye) was employed to extract DNA from all pathogenic bacteria isolates. A template for multiplex polymerase chain reaction (mPCR) was used from the liquid portion that contained DNA. Using an ultraviolet spectrophotometer, the A260/A280 ratio was quantified to evaluate the quality of the DNA, and its integrity was determined through agarose electrophoresis. The isolates mentioned above' genomic DNA (gDNA) was maintained at a temperature of -40 °C until it was defrosted on ice just prior to analysis.

DNA Amplification and Detection

The screening for the following resistance genes was conducted using Multiplex PCR, as previously outlined: Resistance to carbapenems. The mentioned terms include bla_{OXA-23}, bla_{OXA-48}, OmpA [20], [21], and extended spectrum-β-lactamases bla_{TEM} [22]. The following temperature and cycling settings were used (Table 2).

Table (2): Research primers, temperature, and cycles of each gene.

Gene	Sequence (5…….3)*	Annealing temperature	DNA amplicon size (bp	No. of cycles
***OmpA*-F** ***OmpA*-R**	ATTCGAATTCGCTACTATGCTTGTTGCT GCT ATGTAAGCTTCGCTTCTTGACCAGGTTG AAC	60.9	1043	35
bla_{OXA-23}-F **bla_{OXA-23}-R**	GATCGGATTGGAGAACCAGA ATTTCTGACCGCATTTCCAT	56	501	35
bla_{OXA-48}-F **bla_{OXA-48}-R**	GCGTGGTTAAGGATGAACAC CATCAAGTTCAACCCAACCG	52	438	35
bla_{TEM}-F **bla_{TEM}-R**	GAGACAATAACCCTGGTAAAT AGAAGTAAGTTGGCAGCAGTG	56	459	35

X-ray radiation

Sub-culture for bacterial isolates has the MDR and XDR, as well as exposure to two levels of X-ray radiation of the lumbosacral spine. Radiation doses 40(80KV) mAs for 125msec and 51,2(85KV) mAs for 160msec. After that, bacterial isolates examined antimicrobial susceptibility testing.

Statistical analysis

Data was analysed using IBM SPSS-29 (IBM Statistical Packages for Social Sciences, version 29, Chicago, IL, USA). Simple frequency, percentage, mean, standard deviation, and range measurements were used. The significance of percentage differences (qualitative data) was assessed using Pearson Chi-square (x2-test) with Yate's correction or Fisher Exact test when applicable. P values of 0.05 or less indicated statistical significance.

Ethical consideration

The Medical Ethics Committee of the University of Al-Anbar Governorate in Ramadi, Iraq, granted approval for this study in accordance with the Helsinki Declaration. Every individual involved in the research, including patients and their parents, submitted a completed informed permission form (dated 3/12/2023).

3. Results and discussion

Among 120 samples from burn patients admitted to the burn units of Ramadi Teaching Hospital and Falluja Teaching Hospital. After being diagnosed , the numbers of each bacteria were shown in

(Figure 2). This shows that *Pseudomonas aeruginosa* was the most prevalent bacteria comparison to other species.

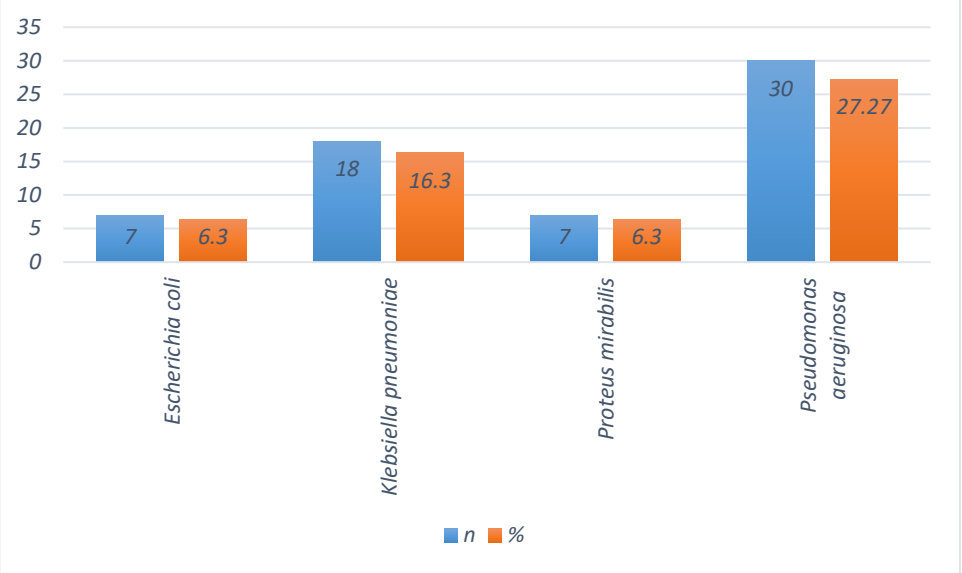

Figure (2): The distribution of bacteria isolated from patients

(Table 3) shows a relationship between the Al-Ramdi unit and the Al-Fallujah unit according to the type of bacterial infections.

Table (3): The percentage of bacteria isolated from burn patients in two units.

Bacteria isolated from patients	**Al-Fallujah center**	**Al-Ramadi center**	**P-value***
	n\%	n\%	
Escherichia coli	4(7.4%)	3(7%)	
Klebsiella pneumoniae	14(20.6%)	4(9.3%)	
Proteus mirabilis	5(7.4%)	2(4.7%)	0.174
Pseudomonas aeruginosa	14(20.6%)	16(37.2%)	

*Significant difference between percentages using Pearson Chi-square test (x^2-test) at 0.05 level.

To identify antibiotic-resistant species, a total of 120 samples were tested. 30(27%) of the isolates was P. aeruginosa, 18(16%) Klebsiella spp, 7(6%) E.coli, 7(6%) P.mirabilis. The Clinical and Laboratory Standards Institute (CLSI) issued guidelines for selecting antibiotics for each strain. The findings demonstrated that Cefepime exhibited a greater proportion of antibiotic resistance at 20(32%), whereas Piperacillin / Tazobactam and Ciprofloxacin had a lower percentage at 18(29%) (Figure 3).

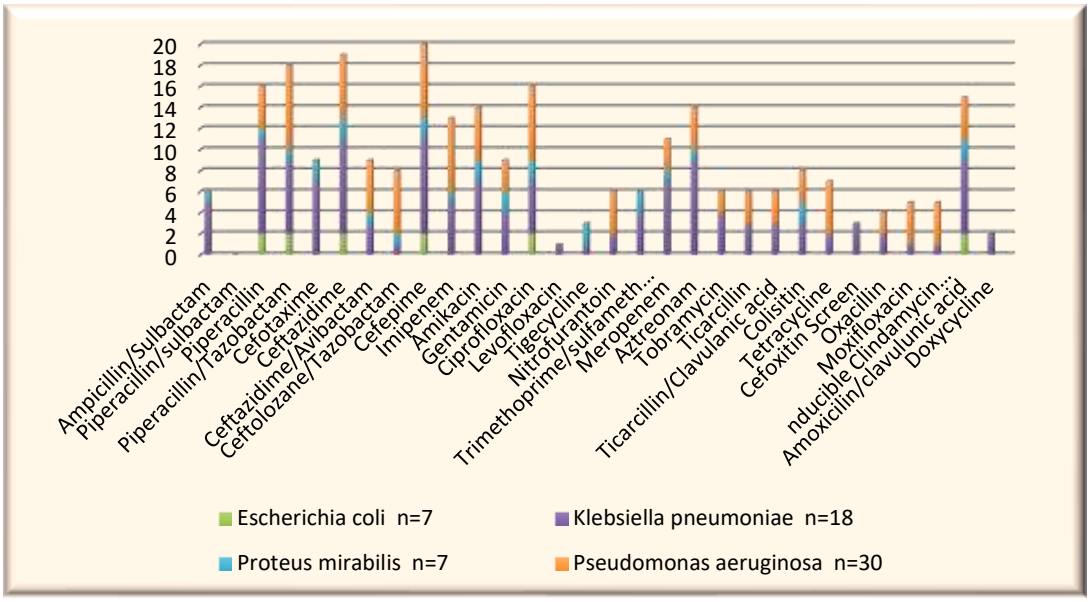

Figure (3): Numbers of antibiotic resistances in bacterial isolates

Bacteria resistant to many antibiotics were discovered in over three categories of drugs. A high percentage of isolates showed multidrug-resistant bacteria(MDR) and Extensively drug-resistant (XDR). All isolates showed high capacity of biofilm formation (Table 4).

Table (4): The percentage of MDR, XDR, and biofilm formation.

Hospital name	**MDR**	**PDR[a]**	**XDR**	**Strongbiofilm formation**	**Moderate biofilm formation**
	n\%	n\%	n\%	n\%	n\%
Al-Fallujah hospital	49(72.1%)	3(4.4%)	16(23.5%)	39(57.4%)	29(24.6%)
AL-Ramadi hospital	28(65.1%)	1(2.3%)	14(32.6%)	25(58.1%)	18(41.9%)
p-value*	0.523			0.935	

Abbreviation: [a] Pan drug resistance

*Significant difference between percentages using Pearson Chi-square test (x^2-test) at 0.05 level.

In 64 (50%) of the isolates, strong biofilm formation was observed. There was no significant difference in the biofilm formation capacity of isolates from the two hospitals, as indicated by the statistical analysis conducted using Pearson's chi-square test (P = 0.935).

Molecular typing was conducted on multi-drug resistant (MDR) and extensively drug resistance (XDR) isolates, consisting of 30 P. aeruginosa, 18 Klebsiella spp, 7 E.coli, 7 P.mirabilis. The results of the Multiplex-PCR analysis indicated that The *bla*$_{TEM}$ gene were the most prevalent, accounting for 33(30%) of the samples. This was followed by OXA-23, which accounted for 27(24.5%), *OmpA* at 25(22.7%), and OXA-48 at 15(13.6%), respectively (Table 5).

The radiation effect of X-rays generated by two doses, 40(80KV)mAs for 125msec and 51,2(85KV) mAs for 160msec, show that there is no effect or change on the resistance ability on all obtained bacterial isolates .

Table (5): Distribution of genes among bacterial pathogen

	P.aeruginosa		***K.pneumoniae***		***E.coli***		***P.mirabilis***	
Gene	n\N	%	n\N	%	n\N	%	n\N	%
OXA-23	(11\30)	36%	(6\18)	33%	(5\7)	71%	(5\7)	28%
OXA-48	(4\30)	13%	(4\18)	22%	(5\7)	71%	(2\7)	28%

OmpA	(0\30)	0%	(15\18)	83%	(4\7)	57%	(6\7)	85%
bla-TEM	(8\30)	26%	(16\18)	53%	(4\7)	42%	(5\7)	71%

*Proportion of gene strains that test positive. n is the count of positive strains for the gene, while N represents the count of strains selected for the test.

Discussion

The mortality rate of burn patients in Iraq has risen significantly in recent years. Burn patients often experience complicated bacterial infections that can lead to septicemia and mortality. Many microbiological infections are caused by multidrug-resistant bacteria, making treatment difficult in hospitals[23].

Pseudomonas aeruginosa has the second-highest rate of isolation among Gram-negative bacteria30(27%), in agreement with previous studies in Algeria[24]. *Pseudomonas aeruginosa* is commonly linked to both hospital-acquired and community-acquired infections. The bacteria identified in patients of the burn unit consistently had the highest numerical value. They can be characterised as a challenging, highly infectious, and widespread pathogen in this particular unit[25].

Enterobacter spp and *Klebsiella pneumoniae*. Comprised 28.9% of all isolates in this investigation, making it one of the most prevalent isolates. Gram-negative homologous anaerobes that are extensively distributed in nature are found within the *Enterobacter* genus. In recent decades, the genus *Enterobacter* has already elicited heightened concern due to its increased association with nosocomial infections[26], [27].

According to Table 1, there is no significant difference between the two centres in terms of the type of bacteria (P = 0.174), which can be attributed to nosocomial outbreaks[28].

According to the susceptibility test of isolates (Table 2), 72.1 % were MDR, 23.5% were XDR, and 4.4% were PDR; this is in agreement with other studies [29], [30].

Molecular typing

The narrow-spectrum beta-lactamases (TEM 1; TEM 2, or SHV 1) genes are the source of ESBLs, which are the result of mutations that alter the amino acid arrangement surrounding the active site. Plasmids are typically responsible for encoding certain bacterial species and are readily transferable between them [31].

The identification of the bla_{TEM} gene at the molecular level in this research may lead to resistance against cephalosporins, such as ceftazidime, cefazolin, cefoxitin, and Cefotaxime. As a result, it is accountable for the formation of phenotypic extended-spectrum β-lactamases [32], [33]. [34]. The TEM type β-lactamases are derived from the enzymes TEM-1 and TEM-2. TEM-1 is a highly significant β-lactamase enzyme that is commonly found in Gram-negative bacteria. More than 130 TEM enzymes have been identified, utilising a valuable mechanism to spread resistant genes [35]. In this study,*K. pneumonia* (83%) and *P. mirabilis* (85%) agreed with a study by Haider et al.[36] (Figure 4). *P.aeruginosa* showed (26%) positive result for the detection of this gene which agreed with a study in Algeria [37]. *E. coli* showed (57%) positive results, which agreed with a study by Haider et[38]. This variation can attributed to the small size of the sample in our study.

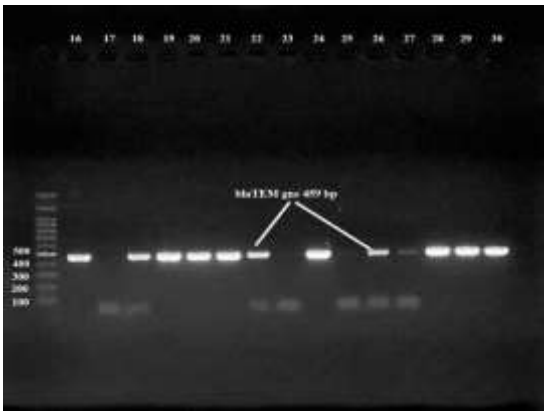

Figure (4): The gene bla-TEM detection in bacterial isolates producing carbapenemase with multiplex PCR.

OmpA is a type of protein called porin, which is found in the outer membrane [39]. OmpA contributes to disease through many mechanisms, including apoptotic induction, immunomodulation, cell adhesion and invasion, biofilm formation, and antimicrobial resistance. OmpA can cause apoptosis in dendritic cells by specifically targeting the mitochondria [40]. In this study, predominant of isolates were positive for the OmpA gene, and all isolates which were forming biofilm formation showed positive OmpA; this may interpret the strong relation between this gene and biofilm formation this agreement with the study performed in Iran. And South Korea [21] (Figure 5).

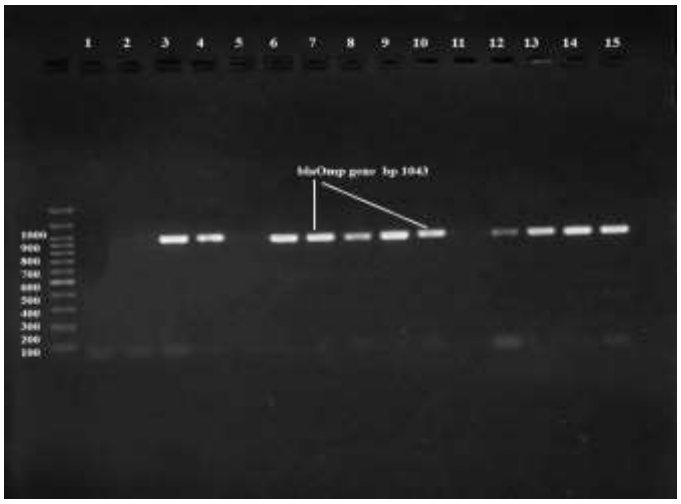

Figure (5): The gene Omp detection in bacterial isolates producing carbapenemase with multiplex PCR.

Carbapenem resistance encompasses the development of various mechanisms, including the production of carbapenemase enzymes, alterations in membrane permeability, changes in penicillin-binding proteins, and enhanced expression of efflux pumps[41], [42]. Among the, OXA-23-like and OXA-24-like carbapenemases have frequently been identified as the most common types in numerous nations[43], [44]. (33%) of *K. pneumonia* show positive for the OXA-23 gene. This may be because of transmission of the gene by plasmid; this finding agreed with a study by Shahid *et al* [45]. *P.aeruginosa* showed (36%) positive for the OXA-23 gene (Figure 6).

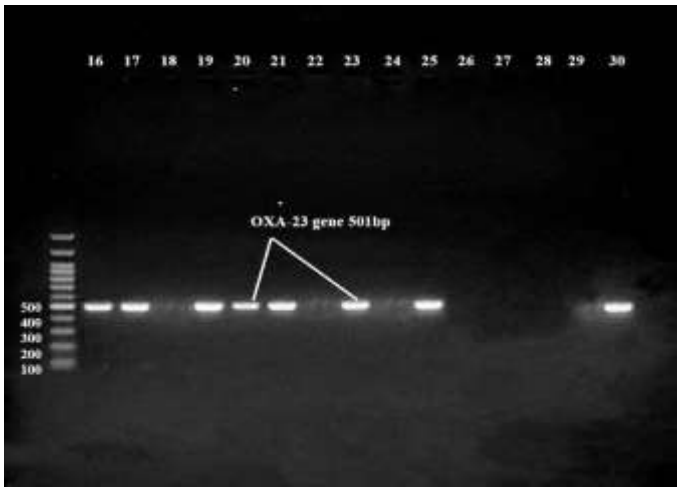

Figure (6): The gene OXA-23 detection in bacterial isolates producing carbapenemase with multiplex PCR.

And -24/40 cause resistance to carbapenem antibiotics such as imipenem (IMP), meropenem (MEM), and doripenem (DRP)[46]. Studies on the prevalence of carbapenem-resistant *Pseudomonas aeruginosa* (CRPA) have shown estimates ranging from 10% to 50% worldwide. Brazil, Peru, Costa Rica, Russia, Greece, Poland, Iran, and Saudi Arabia all have CRPA rates exceeding 50%, while the Dominican Republic and Canada have the lowest rates at 3.3% and 8%, respectively. In recent years, CRPA's geographic reach has been expanding[47]. As has been observed in other bacterial species, the transfer of genetic material through mobile genetic elements is one potential scenario for the acquisition of the plasmid-mediated bla_{OXA-23}.

The appearance of the OXA (oxacillinase) group of β-lactamases (Class D) has led to several challenges in managing and treating opportunistic infections. The bla_{OXA-48} gene is highly prevalent in *K. pneumoniae*, and *Enterobacteriaceae* serves multiple crucial functions, including the facilitation of biofilm formation and the development of resistance against carbapenems [48], [49]. To date, the bla_{OXA-48} gene has been identified in 4(13%) *P. aeruginosa isolates*.These findings indicated that the prevalence of bla_{OXA-48} may differ among countries (Figure 7).

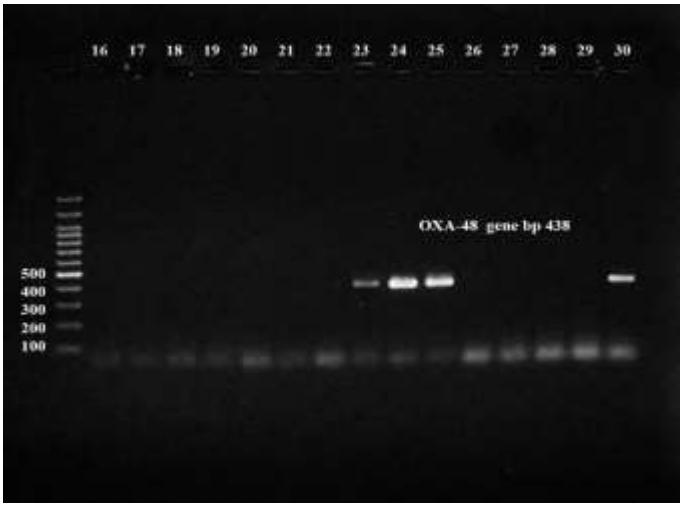

Figure (7): The gene OXA-48 detection in *K. pneumoniae* producing carbapenemase with multiplex PCR.

Biofilm formation

Bacterial biofilm formation is a significant pathogenicity factor that inhibits the effectiveness of antibiotics and immune defence responses[50], [51]. Bacteria have acquired numerous virulence factors and are accountable for causing severe, life-threatening infections[52]. The ability to form

biofilms and the presence of diverse adhesins contribute to the development of infections and resistance to antimicrobial medicines[53]. In this study, bacteria isolates show the formation of biofilm in all isolates, in agreement with two studies performed in Iraq[54], [55].

Benefits of X-ray radiation

This study showed that the exposition of bacterial cultures to X-ray dose has not changed the susceptibility to antibiotics. This disagreement with the study performed in Tunis, which find the susceptibility of bacteria decreases after exposure [56]. Also, the two studies in Iraq show that routine diagnostic radiation X-ray has been discovered to have a significant impact on the equilibrium of microbial populations in the oral flora by raising the density of certain types of microorganisms[14], [15]. On the other hand, this result agreed with other studies [57], [58]. This attribute These isolates demonstrated cellular adaptability when exposed to clinical X-ray irradiation, hence improving their capacity to survive in the radiology department. This flexibility is facilitated by the isolate's capacity to produce a significant amount of catalase, which assists in mitigating the detrimental impact of the oxidative stress caused by these therapeutic radiations.

4. Conclusion and future scope

This study demonstrated that the isolates obtained from the burn unit are ESBL-producing, indicating a significant concern in the intensive care unit due to the high morbidity associated with it, which might potentially result in fatalities. These findings are consistent with the phenotypic test results for antibiotics, which revealed a high level of resistance to cefepime. All isolates showing high ability to form biofilms, which is an important virulence factor. This requires immediate action to effectively manage any outbreak of nosocomial illness. No significant effect of X-ray on a bacterial isolate susceptibility from the burn unit was detected.

Limitations of study

The study encompassed patients from the Burn Unit of the Al-Anbar Government, which were required to be obtained from units in all burn centers throughout Iraq. Additionally, the duration of the investigation may be brief; therefore, we recommend that the study be extended to accommodate a greater number of samples. Our study focuses on two doses of X-ray .It may need to use more than two dose at different duration

Reference

[1] I. E. Mba and E. I. Nweze, “Nanoparticles as therapeutic options for treating multidrug-resistant bacteria: research progress, challenges, and prospects,” *World J. Microbiol. Biotechnol.*, vol. 37, no. 6, p. 108, 2021, doi: 10.1007/s11274-021-03070-x.

[2] R. Vivas, A. A. T. Barbosa, S. S. Dolabela, and S. Jain, “Multidrug-resistant bacteria and alternative methods to control them: an overview,” *Microb. Drug Resist.*, vol. 25, no. 6, pp. 890–908, 2019.

[3] F. N. Williams and J. O. Lee, “Pediatric burn infection,” *Surg. Infect. (Larchmt).*, vol. 22, no. 1, pp. 54–57, 2021.

[4] G. Haidar *et al.*, “Ceftolozane-tazobactam for the treatment of multidrug-resistant Pseudomonas aeruginosa infections: clinical effectiveness and evolution of resistance,” *Clin. Infect. Dis.*, vol. 65, no. 1, pp. 110–120, 2017.

[5] M. T. Moghadam, A. Shariati, S. Mirkalantari, and A. Karmostaji, “The complex genetic region conferring transferable antibiotic resistance in multidrug-resistant and extremely drug-resistant Klebsiella pneumoniae clinical isolates,” *New Microbes New Infect.*, vol. 36, p. 100693, 2020.

[6] R. Ranjbar and A. Farahani, “Study of genetic diversity, biofilm formation, and detection of Carbapenemase, MBL, ESBL, and tetracycline resistance genes in multidrug-resistant Acinetobacter baumannii isolated from burn wound infections in Iran,” *Antimicrob. Resist. Infect. Control*, vol. 8, pp. 1–11, 2019.

[7] A. Rehman, W. M. Patrick, and I. L. Lamont, "Mechanisms of ciprofloxacin resistance in Pseudomonas aeruginosa: new approaches to an old problem," *J. Med. Microbiol.*, vol. 68, no. 1, pp. 1–10, 2019, doi: https://doi.org/10.1099/jmm.0.000873.

[8] L. Serwecińska, "Antimicrobials and antibiotic-resistant bacteria: a risk to the environment and to public health," *Water*, vol. 12, no. 12, p. 3313, 2020.

[9] S. Natoubi *et al.*, "Epidemiology of extended-spectrum β-lactamases and carbapenemases producing Enterobacteriaceae in Morocco," *J Contemp Clin Pr.*, vol. 6, no. 2, pp. 75–85, 2020.

[10] D. Aruhomukama *et al.*, "Bla VIM-and bla OXA-mediated carbapenem resistance among Acinetobacter baumannii and Pseudomonas aeruginosa isolates from the Mulago hospital intensive care unit in Kampala, Uganda," *BMC Infect. Dis.*, vol. 19, pp. 1–8, 2019.

[11] F. Longo, C. Vuotto, and G. Donelli, "Biofilm formation in Acinetobacter baumannii," *New Microbiol*, vol. 37, no. 2, pp. 119–127, 2014.

[12] A. F. Tawfik, A. M. Alswailem, A. M. Shibl, and M. H. M. Al-Agamy, "Prevalence and genetic characteristics of TEM, SHV, and CTX-M in clinical Klebsiella pneumoniae isolates from Saudi Arabia," *Microb. drug Resist.*, vol. 17, no. 3, pp. 383–388, 2011.

[13] L. Poirel, T. Naas, and P. Nordmann, "Diversity, epidemiology, and genetics of class D β-lactamases," *Antimicrob. Agents Chemother.*, vol. 54, no. 1, pp. 24–38, 2010.

[14] A. A. H. S. Al-Janabi and M. H. H. AI-Baghdadi, "Evaluation of biological effects of X-radiography and computed tomography scan on oral microflora," *Int. J. Oral Heal. Sci.*, vol. 9, no. 1, pp. 20–24, 2019.

[15] F. H. Kamel and A. I. Abdi, "Survival of oral bacterial after X-rays diagnosis," 2019.

[16] J. M. Andrews, "Determination of minimum inhibitory concentrations.," *J. Antimicrob. Chemother.*, vol. 48 Suppl 1, pp. 5–16, Jul. 2001, doi: 10.1093/jac/48.suppl_1.5.

[17] A. Zapata and S. Ramirez-Arcos, "A comparative study of McFarland turbidity standards and the Densimat photometer to determine bacterial cell density.," *Curr. Microbiol.*, vol. 70, no. 6, pp. 907–909, Jun. 2015, doi: 10.1007/s00284-015-0801-2.

[18] P. A. Wayne, "Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing," 2011.

[19] S. Kırmusaoğlu, "The Methods for Detection of Biofilm and Screening Antibiofilm Activity of Agents," S. Kırmusaoğlu, Ed., Rijeka: IntechOpen, 2019, p. Ch. 6. doi: 10.5772/intechopen.84411.

[20] K. Jalalvand, N. Shayanfar, F. Shahcheraghi, E. Amini, M. Mohammadpour, and P. Babaheidarian, "Evaluation of phenotypic and genotypic characteristics of carbapnemases-producing enterobacteriaceae and its prevalence in a referral hospital in Tehran city," *Iran. J. Pathol.*, vol. 15, no. 2, p. 86, 2020.

[21] F. Badmasti, S. D. Siadat, S. Bouzari, S. Ajdary, and F. Shahcheraghi, "Molecular detection of genes related to biofilm formation in multidrug-resistant Acinetobacter baumannii isolated from clinical settings," *J. Med. Microbiol.*, vol. 64, no. Pt_5, pp. 559–564, 2015.

[22] R. K. Sah, P. Dahal, R. Parajuli, G. R. Giri, and E. Tuladhar, "Prevalence of bla CTX-M and bla TEM Genes in Cefotaxime-Resistant Escherichia coli Recovered from Tertiary Care at Central Nepal: A Descriptive Cross-Sectional Study," *Can. J. Infect. Dis. Med. Microbiol.*, vol. 2024, 2024.

[23] T. A. Kadhim *et al.*, "The Bacterial Contamination in the Burn Unit of Al-Hussain Teaching Hospital at Al-Samawa City, Iraq," *Med. J. Babylon*, vol. 20, no. 2, pp. 357–361, 2023.

[24] A. Chaibdraa and M. C. Bentakouk, "[Not Available].," *Ann. Burns Fire Disasters*, vol. 21, no. 1, pp. 7–12, Mar. 2008.

[25] H. K. Hasan and A. K. Al-Humairi, "Sociodemographic characteristics and fate of hospitalized burned patients in Al-Hilla city," *Med. J. Babylon*, vol. 19, no. 4, pp. 547–553, 2022.

[26] S. Santajit and N. Indrawattana, "Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens.," *Biomed Res. Int.*, vol. 2016, p. 2475067, 2016, doi: 10.1155/2016/2475067.

[27] O. A. Forson, E. Ayanka, M. Olu-Taiwo, P. J. Pappoe-Ashong, and P. J. Ayeh-Kumi, "Bacterial infections in burn wound patients at a tertiary teaching hospital in Accra, Ghana," *Ann. Burns Fire Disasters*, vol. 30, no. 2, p. 116, 2017.

[28] A.-L. Munier *et al.*, "Incidence, risk factors and outcome of multi-drug resistant Acinetobacter baumannii nosocomial infections during an outbreak in a burn unit," *Int. J. Infect. Dis.*, vol. 79, pp. 179–184, 2019.

[29] A. M. Lachiewicz, C. G. Hauck, D. J. Weber, B. A. Cairns, and D. van Duin, "Bacterial infections after burn injuries: impact of multidrug resistance," *Clin. Infect. Dis.*, vol. 65, no. 12, pp. 2130–2136, 2017.

[30] H. Kanamori *et al.*, "A Prolonged Outbreak of KPC-3-Producing Enterobacter cloacae and Klebsiella pneumoniae Driven by Multiple Mechanisms of Resistance Transmission at a Large Academic Burn Center.," *Antimicrob. Agents Chemother.*, vol. 61, no. 2, Feb. 2017, doi: 10.1128/AAC.01516-16.

[31] J. Sharma, M. Sharma, and P. Ray, "Detection of TEM & SHV genes in Escherichia coli & Klebsiella pneumoniae isolates in a tertiary care hospital from India.," *Indian J. Med. Res.*, vol. 132, pp. 332–336, Sep. 2010.

[32] E. J. Alyamani, M. A. Khiyami, R. Y. Booq, B. M. Alnafjan, M. A. Altammami, and F. S. Bahwerth, "Molecular characterization of extended-spectrum beta-lactamases (ESBLs) produced by clinical isolates of Acinetobacter baumannii in Saudi Arabia," *Ann. Clin. Microbiol. Antimicrob.*, vol. 14, pp. 1–9, 2015.

[33] A. A. K. Shali, P. J. Jalal, and S. K. Arif, "Research Article Dissemination and Genetic Relatedness of Multidrug-Resistant and Extensively Drug-Resistant Acinetobacter baumannii Isolates from a Burn Hospital in Iraq," 2022.

[34] M. M. Feizabadi *et al.*, "Distribution of bla TEM, bla SHV, bla CTX-M genes among clinical isolates of Klebsiella pneumoniae at Labbafinejad Hospital, Tehran, Iran," *Microb. drug Resist.*, vol. 16, no. 1, pp. 49–53, 2010.

[35] N. M. Alkasaby and M. El Sayed Zaki, "Molecular Study of Acinetobacter baumannii Isolates for Metallo-β-Lactamases and Extended-Spectrum-β-Lactamases Genes in Intensive Care Unit, Mansoura University Hospital, Egypt," *Int. J. Microbiol.*, vol. 2017, no. 1, p. 3925868, 2017.

[36] M. H. Haider, N. U. Ain, S. Abrar, and S. Riaz, "blaOXA, blaSHV-, and blaTEM-extended-spectrum β-lactamases in Gram-negative strains from burn patients in Lahore, Pakistan," *J. Infect. Dev. Ctries.*, vol. 14, no. 12, pp. 1410–1417, 2020.

[37] A. Tchakal-Mesbahi, M. Metref, V. K. Singh, M. Almpani, and L. G. Rahme, "Characterization of antibiotic resistance profiles in Pseudomonas aeruginosa isolates from burn patients," *Burns*, vol. 47, no. 8, pp. 1833–1843, 2021.

[38] M. H. Haider, T. D. McHugh, K. Roulston, L. B. Arruda, Z. Sadouki, and S. Riaz, "Detection of carbapenemases bla OXA48-bla KPC-bla NDM-bla VIM and extended-spectrum-β-lactamase bla OXA1-bla SHV-bla TEM genes in Gram-negative bacterial isolates from ICU burns patients," *Ann. Clin. Microbiol. Antimicrob.*, vol. 21, no. 1, p. 18, 2022.

[39] J. A. Gaddy and L. A. Actis, "Regulation of Acinetobacter baumannii biofilm formation," 2009.

[40] J. S. Lee, C. H. Choi, J. W. Kim, and J. C. Lee, "Acinetobacter baumannii outer membrane protein A induces dendritic cell death through mitochondrial targeting.," *J. Microbiol.*, vol. 48, no. 3, pp. 387–392, Jun. 2010, doi: 10.1007/s12275-010-0155-1.

[41] C. Héritier, L. Poirel, T. Lambert, and P. Nordmann, "Contribution of acquired carbapenem-hydrolyzing oxacillinases to carbapenem resistance in Acinetobacter baumannii," *Antimicrob. Agents Chemother.*, vol. 49, no. 8, pp. 3198–3202, 2005.

[42] M. S. Amudhan, U. Sekar, A. Kamalanathan, and S. Balaraman, "blaIMP and blaVIM mediated carbapenem resistance in

Pseudomonas and Acinetobacter species in India," *J. Infect. Dev. Ctries.*, vol. 6, no. 11, pp. 757–762, 2012.

[43] R. Zarrilli, S. Pournaras, M. Giannouli, and A. Tsakris, "Global evolution of multidrug-resistant Acinetobacter baumannii clonal lineages," *Int. J. Antimicrob. Agents*, vol. 41, no. 1, pp. 11–19, 2013.

[44] L. Thabet, A. Turki, S. Ben Redjeb, and A. allah Messadi, "[Bacteriological profile and antibiotic resistance of bacteria isolates in a burn department].," *Tunis. Med.*, vol. 86, no. 12, pp. 1051–1054, Dec. 2008.

[45] M. Shahid *et al.*, "Clinical carbapenem-resistant Klebsiella pneumoniae isolates simultaneously harboring bla (NDM-1), bla (OXA) types and qnrS genes from the Kingdom of Bahrain: Resistance profile and genetic environment.," *Front. Cell. Infect. Microbiol.*, vol. 12, p. 1033305, 2022, doi: 10.3389/fcimb.2022.1033305.

[46] N. Woodford *et al.*, "Multiplex PCR for genes encoding prevalent OXA carbapenemases in Acinetobacter spp.," *Int. J. Antimicrob. Agents*, vol. 27, no. 4, pp. 351–353, Apr. 2006, doi: 10.1016/j.ijantimicag.2006.01.004.

[47] D. J. Hong, I. K. Bae, I.-H. Jang, S. H. Jeong, H.-K. Kang, and K. Lee, "Epidemiology and Characteristics of Metallo-β-Lactamase-Producing Pseudomonas aeruginosa.," *Infect. Chemother.*, vol. 47, no. 2, pp. 81–97, Jun. 2015, doi: 10.3947/ic.2015.47.2.81.

[48] L. Poirel, A. Potron, and P. Nordmann, "OXA-48-like carbapenemases: the phantom menace," *J. Antimicrob. Chemother.*, vol. 67, no. 7, pp. 1597–1606, 2012.

[49] J. Ramos-Vivas *et al.*, "Biofilm formation by multidrug resistant Enterobacteriaceae strains isolated from solid organ transplant recipients," *Sci. Rep.*, vol. 9, no. 1, p. 8928, 2019.

[50] R. M. Abd El-Baky, T. Sandle, J. John, G. E.-D. A. Abuo-Rahma, and H. F. Hetta, "A novel mechanism of action of ketoconazole: inhibition of the NorA efflux pump system and biofilm formation in multidrug-resistant Staphylococcus aureus.," *Infect. Drug Resist.*, vol. 12, pp. 1703–1718, 2019, doi: 10.2147/IDR.S201124.

[51] M. A. E.-G. El-Sayed Ahmed, L.-L. Zhong, C. Shen, Y. Yang, Y. Doi, and G.-B. Tian, "Colistin and its role in the Era of antibiotic resistance: an extended review (2000-2019).," *Emerg. Microbes Infect.*, vol. 9, no. 1, pp. 868–885, Dec. 2020, doi: 10.1080/22221751.2020.1754133.

[52] H. Aydemir, H. I. Tuz, N. Piskin, G. Celebi, C. Kulah, and F. Kokturk, "Risk factors and clinical responses of pneumonia patients with colistin-resistant Acinetobacter baumannii-calcoaceticus.," *World J. Clin. cases*, vol. 7, no. 10, pp. 1111–1121, May 2019, doi: 10.12998/wjcc.v7.i10.1111.

[53] R. M. Abdullah and R. Z. Ahmed, "Genotype detection of fimH gene of Acinetobacter baumannii isolated from different clinical cases," *J. Biotechnol. Res. Cent.*, vol. 13, no. 1, pp. 23–28, 2019.

[54] I. M. S. Al-Kadmy, A. N. M. Ali, I. M. A. Salman, and S. S. Khazaal, "Molecular characterization of Acinetobacter baumannii isolated from Iraqi hospital environment," *New microbes new Infect.*, vol. 21, pp. 51–57, 2018.

[55] P. A. Hassan and A. K. Khider, "Correlation of biofilm formation and antibiotic resistance among clinical and soil isolates of Acinetobacter baumannii in Iraq," *Acta Microbiol. Immunol. Hung.*, pp. 1–10, 2019.

[56] J. Cherif, A. Raddaoui, M. Trabelsi, and N. Souissi, "Diagnostic low-dose X-ray radiation induces fluoroquinolone resistance in pathogenic bacteria," *Int. J. Radiat. Biol.*, vol. 99, no. 12, pp. 1971–1977, 2023.

[57] K. Hayashi *et al.*, "Exposure to clinical X-ray radiation does not alter antibiotic susceptibility or genotype profile in gram-negative and gram-positive clinical pathogens.," *Br. J. Biomed. Sci.*, vol. 69, no. 3, pp. 119–122, 2012.

[58] K. S. Adebiyi, F. O. Ehigiamusoe, and C. E. Oshoma, "Effect of Clinical X-rays on Methicillin-resistant Staphylococcus aureus and Other Bacterial Isolates from Radiology Equipment," 2020.