DOI: https://doi.org/10.53350/pjmhs2023174235

ORIGINAL ARTICLE

Detection of B-Lactamase OXA Genes and Biofilm Production in Carbapenem Resistant Gram-Negative Bacilli

AMNA SARWAR[1], SYEDA ZAHRA GILLANI[2], RANA MUHAMMAD SHAHBAKHT[3], FIZA SHAFQAT[4], AJMAL NISAR[5], SIDRA MENGAL[6], ABEERA ASHRAF[7], ZIAN ASIF[8]
[1,2]Faculty of Life Sciences, University of Central Punjab, Lahore, Pakistan
[3]Faculty of Veterinary & Animal Sciences, Animal Feed & Production, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan
[4]Institute of Microbiology and Molecular Genetics, University of The Punjab, Lahore, Pakistan
[5]Department of Clinical Medicine, University of Veterinary and Animal Sciences, Lahore
[6]Department of Microbiology, University of Balochistan, Pakistan.
[7]Faculty of Veterinary Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan
[8]Department of Biomedical Sciences, Microbiology and clinical Microbiology, Humanitas University, Via Rita Levi Montalcini 4, 20072 Pieve Emanuele- Milan, Italy.
Correspondence to: Rana Muhammad Shahbakht, Email: muhammad.shahbakht@mnsuam.edu.pk

ABSTRACT

Objectives: The emergence of carbapenem resistance in biofilm producer Gram-negative bacteria poses a substantial challenge to manage infections. This study aimed to evaluate biofilm production and molecular characterization of carbapenem resistance Gram-negative rods.

Methods: Total of 46 bacteria were isolated from various clinical samples, including blood, CSF, urine, sputum, saliva, tracheal secretion, and pus. Following the standard guidelines for bacterial identification (CLSI), bacterial pathogens are isolated and identified. The antibiotic susceptibility profile was obtained against commercially available antibiotics including Amoxicillin, Amikacin, Ceftazidime, Cefepime, Imipenem, Meropenem, Gentamycin, Tobramycin, Doxycycline, Minocycline, Tazobactam, Ciprofloxacin, and Levofloxacin following CLSI guidelines. Biofilm production potential was quantified by using 96-well flat bottom microtiter plates. Phenotypic and molecular characterization of carbapenem-resistant bacteria was performed. The genotypic resistance to carbapenem was determined using OXA-23, OXA-24, OXA-51 and OXA-58 gene primers.

Results: The Gram-negative bacterial strains were found as Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, and Serratia marcescens. The phenotypic resistance against carbapenem drugs was 95%. DNA of carbapenem-resistant bacteria isolated by commercially available kit and polymerase chain reaction assay was performed and optimized to identify OXA-23 (84%), OXA-24(90%), OXA-51(83%) and OXA-58(80%) gene in bacterial strains.

Practical Implication: For better and prompt management of antimicrobial resistance further work on genetics is required and warranted to produce gene effective drugs to enhance antimicrobial effect of carbapenems.

Conclusion: The prevalence of carbapenem resistance among Gram-negative bacilli was high. OXA genes have raised clinical concern and further study is required to detect carbapenemase-producing bacteria.

Keywords: Antibiotic Resistance, Biofilm, Carbapenem-resistance, Escherichia coli, Acinetobacter baumannii, Klebsiella pneumoniae, Serratia marcescens, β-lactamases, OXA-23, OXA-24, OXA-51 and OXA-58.

INTRODUCTION

Antimicrobial resistance (AMR) poses a high health risk and becomes a serious global health threat of the 21st century [1]. Biofilm promotes the transfer of antimicrobial resistance genes among bacteria and enhances bacterial tolerance to environmental stress. Misuse and overuse of antibiotics gives rise to the critical trial to public health in expanding antibiotic resistance and its corresponding effect on diseases, death rates, and increasing adverse after-effects. Biofilms are a major health concern because of their ability to resist antibiotics and tolerate external stress, therefore causing persistent chronic bacterial infection [2].Antibiotic resistance in Gram-negative rods has been increasing along with reduced effective antibiotics and the unavailability of inventive ones. This issue will lead to remarkable clinical and economic outcomes such as prolonged treatments, overprized antibiotics, and increased mortality rates [3].

Antimicrobial resistance develops when bacteria evade the mode of action of antibiotics through different mechanisms, like restricting the attachment of the drug to the bacteria, altering their structure, expelling the drug outside the cell and invalidating the effects of antibiotics. The member of Enterobacteriaceae family such as Escherichia coli, Klebsiella spp. and Enterobacter spp., has become a common cause of pneumonia, septicemia, UTIs, and nosocomial infections. They transfer genetic elements through horizontal gene transfer (HGT), frequently intervened by transposons and plasmids[4]. Some new resistance mechanisms are also emerging and causing multidrug resistance (MDR).

Biofilm forming ability and acquired antibiotic resistance genes are mostly responsible for the non-effective behavior of bacteria against an antimicrobial agent [5]. Clinically, persistent and chronic infections are related to biofilms due to the inherent antimicrobial resistance and phenotypic variants [6]. Common biofilm producing bacteria are Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Streptococcus viridans, Staphylococcus aureus, Pseudomonas aeruginosa and Proteus mirabilis[7]. Biofilm formers cause acute and chronic infections including urinary tract, respiratory tract, and gastrointestinal infections [8]. Biofilm forming bacteria have the ability of intrinsic antibiotic resistance and cause multi drug resistant infections in immuno-compromised individuals.[9].

Carbapenems are the only effective antimicrobial agent for the majority of infections caused by Gram-negative bacteria due to their resistance against penicillinases, beta-lactamases and cephalosporinases. The overuse of carbapenems in recent years has caused the development of resistance, which is moderated by carbapenemases [10]. Carbapenem resistance is interceded by two methods; formation of β-lactamase (activated cephalosporinase or ESBL) with carbapenemase activity merged with reduced permeability because of loss or change in porin (b) formation of carbapenem-hydrolyzing β-lactamases [11].

WHO has classified carbapenem-resistant Enterobacteriaceae (CRE) and Acinetobacter baumannii (CRAB) in the perilous priority pathogens group [3]. In Enterobacteriaceae carbapenem-resistance has been mainly reported in Acinetobacter baumannii, Pseudomonas aeruginosa and Klebsiella pneumoniae. These pathogens are responsible for several nosocomial or community-acquired infections like septicemia, peritonitis, UTIs, pneumonia, and meningitis.

Infection with Carbapenem Resistant Enterobacteriaceae (CRE) is an emerging challenge worldwide. The main reason for this issue is mobile carbapenemase enzymes, the most potent beta-lactamases which are categorized into three molecular classes; Ambler (class A, class C and class D) according to the Ambler Classification system. Class A has serine on the active site which can be repressed by β-lactamase inhibitors. It is recognized as Klebsiella-producing carbapenemase [KPC]. Ambler class B

termed Metallo-β-lactamase has zinc on active site and reacts with DPA and EDTA. Class D has serine on its active site, and is suppressed by β-lactamase inhibitors.

The number of carbapenem-resistant biofilm-producing bacteria is increasing and becoming a serious concern around the globe. Biofilms are linked with dramatic changes (physiological) leading to unique properties as compared to planktonic bacteria. The investigation of phenotypes associated with biofilm is a critical first step toward reducing the harmful consequences of pathogenic biofilms. The current study aims to narrate the morphological, biochemical and molecular characteristics of biofilm producing carbapenem resistant Gram-negative bacteria and find the prevalence of bla-OXA genes in the isolated bacterial strains.

MATERIALS AND METHODS

Sample Collection: Total 200 clinical samples were collected aseptically following standard sample collection protocols including blood, CSF, saliva, tracheal secretions, urine, sputum etc. Collected samples were then brought to the Microbiology Lab at Faculty of Life Sciences, University of Central Punjab.

Isolation of Bacterial Isolates: The samples were processed to isolate various bacterial strains through the identification procedure. MacConkey agar and Nutrient agar were used for bacterial isolation. After culturing, plates were placed in an incubator at 37° C for 24 hours. After the overnight incubation, bacterial growth was examined and the colony appearance was noted.

Identification and Confirmation of Bacteria: The bacteria were identified using the macroscopic features; microscopy and biochemical tests were performed to confirm the bacteria. Bacterial isolates were confirmed at the molecular level by using Polymerase reaction (PCR).

Macroscopic identification was done by observing the morphological characteristics including color, size, shape, margins, and elevations. Microscopic identification was done by Gram staining. Isolates were further confirmed by performing biochemical tests including Catalase, Oxidase, Citrate, Urease, Indole and TSI.

Antimicrobial Susceptibility Test: Disc diffusion test by the Kirby-Bauer was performed to evaluate the antimicrobial susceptibility pattern of bacteria according to CLSI. All bacterial isolates were checked for the antibiotic susceptibility pattern of commercialized antibiotic discs. The bacterial suspension was prepared and bacterial culture was collected with bacterial swabs. The swabs were then streaked on Mueller–Hinton plates at 90° angles. Discs were applied followed by overnight incubation at 37 °C and diameters of zone of inhibition were recorded in millimeters (mm) according to CLSI guideline 2017.

Table 1: Primer sequence of the genes used for molecular characterization

Sr. No.	Gene	Primer Sequence	Product size	Reference
1.	blaOXA-23-F	GATCGGATTG GAGAACCAGA	501bp	(Woodford et al., 2006)
	blaOXA-23-R	CTAGCCTAAC CTCTTGGTCT		
2.	blaOXA-24-F	GGTTAGTTGG CCCCCTTAAA	246 bp	(Woodford et al., 2006)
	blaOXA-24-R	CCAATCAACC GGGGGAATTT		
3.	blaOXA-51-F	TAATGCTTTGA TCGGCCTTG	353 bp	(Woodford et al., 2006)
	blaOXA-51-R	ATTACGAAACT AGCCGGAAC		
4.	blaOXA-58-F	AAGTATTGGG GCTTCTGCTG	599 bp	(Woodford et al., 2006)
	blaOXA-58-R	TTCATAACCCC GAAGACGAC		

Molecular characterization: Genomic DNA Isolation: DNA extraction of bacterial cultures was done with commercially available thermos-scientific DNA Extraction Kit (GeneJET Genomic DNA Purification Kit). To confirm the DNA extracted from bacterial strains, gel electrophoresis was performed.

Polymerase Chain Reaction: After DNA extraction, PCR was performed to amplify the primer sequence of the blaOXA-23, blaOXA-24, OXA-51 and OXA-58 genes. To confirm the amplified PCR products, agarose gel was run. The primer sequences are mentioned in the table below. (Table 1)

Biofilm Assay: Biofilm microtiter plate assay was performed on all the bacterial isolates with a modified method using a 96-well flat-bottom plate to evaluate biofilm production potential of bacterial strains. The freshly prepared nutrient broth was dispensed 200µl in all the wells of the plate. A loop full of bacterial growth was inoculated. Last column was left non-inoculated as negative control. Then plate was incubated for 24 hours at 37℃ and OD was taken at 600 nm. The opacity of bacterial suspensions was standardized with 0.5 McFarland (10^8 CFU/ml). For comparing the inoculums, 0.5 McFarland solution was prepared by mixing Sulphuric acid (H_2SO_4) and Barium chloride ($BaCl_2$) and its OD was taken at a wavelength of 600nm. The absorbance value of 0.5McFarland standard was ~0.064 and compared with the bacterial inoculums. After the incubation and comparison, the plate was dumped to discard the unattached cells. Fixation was performed by drying the plate at 60℃ for 1 hr. The washing was performed with PBS twice and the wells were dried again at 60℃ for 1 hour. The 150µl of 0.1% Safranin was added to the wells of the flat bottom 96 wells microtiter plate followed by 15 minutes incubation of the plate at room temperature. The microtiter plate was again washed with PBS twice by turning the plate upside down to perform washing and air-dried the plate. The 150µl of 95% ethanol was added to all wells to re-suspend the biofilm lined with the wells. The optical density was noted by the spectrophotometer at 600 nm and readings were noted.

RESULTS

Out of 200 samples, 46 samples which included pus samples (n=13), wound swabs (n=3), urine samples (n=11), blood samples (n=5), tissue samples (n=2), sputum samples (n=3), tracheal secretions (n=2), tracheal swab (n=7) samples were Gram-negative bacteria. (Table 1)

Table 1: Number of Positive Growths of Gram-Negative Bacteria from Samples

Serial no:	Sample source	No. of samples	Gram neg. bacteria
1	Pus	20	13
2	Wound swab	26	3
3	Urine	38	11
4	Blood	30	5
5	Tissue	19	2
6	Sputum	26	3
7	Tracheal secretion	15	2
8	Tracheal swab	26	7
	Total	200	46

MacConkey agar plates were used to grow bacteria on them. Some bacteria formed yellow, smooth, shiny, and mucoid colonies, few formed dry, donut-shaped, and dark pink color colonies while others produced large, mucoid, dark pink colonies on MacConkey agar. Gram-stained colonies were observed under a microscope in 100X resolution. Few colonies appeared as Gram-negative rods and some were Gram-negative coccobacilli in shape. After obtaining the results of biochemical tests 34 (74%) Acinetobacter baumannii, 8 (17%) Klebsiella pneumoniae, 2 (0.04%) E. coli and 2 (0.04%) Serratia marcescens were identified and confirmed. (Figure 1)

The isolated bacteria were (31.91%) resistant to amoxicillin, (70.72%) resistant to tazobactam, (48.93%) resistant to cefotaxime, (46.80%) resistant to ceftriaxone, (95%) resistance to meropenem and imipenem, (68,08%) resistant to amikacin, (59.57%) resistant to gentamycin, (46.80%) resistant to tobramycin, (36.17%) to doxycycline, (24.25%) to minocycline, (59.57%) to ciprofloxacin, (53.19%) to levofloxacin. (Figure2)

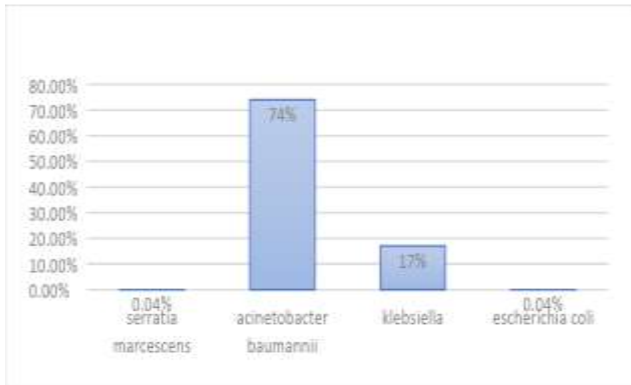

Figure 1: Percentage of Isolated Bacteria from Clinical Samples

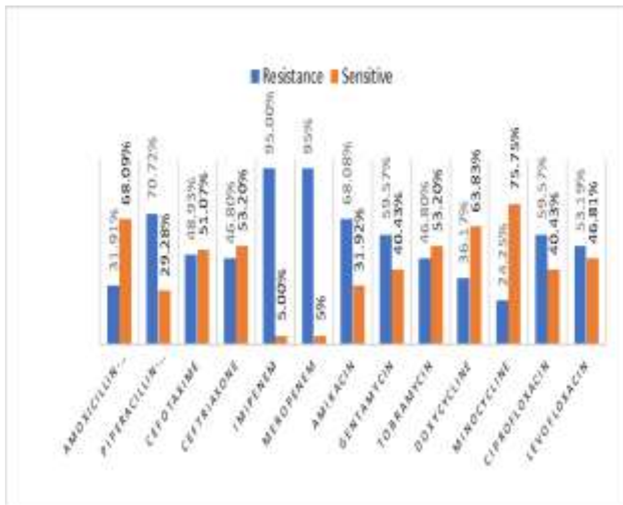

Figure 2: Antibiotic Susceptibility Pattern of Acinetobacter Baumannii, Klebsiella Pneumoniae, E. Coli, Serratia

Bacterial strains were amplified using OXA gene primers to confirm the carbapenem resistance. Samples were selected randomly for molecular characterization. PCR performed on blaOXA-23, blaOXA-24, blaOXA-51, and blaOXA-58 gene primers on 55°C, 54°C, 53°C and 57.5°C respectively. The amplified samples were loaded in the 1.5% gel for visualizing the amplified DNA bands using the 1kb DNA ladder as a reference. The product size of the primers was 501bp, 246bp, 353bp and 599bp respectively. The genomic resistance was found in most samples.

Table 2: Levels of Biofilm Production of Bacterial Isolates

Bacterial isolates (n=46)	Non-biofilm Producers	Biofilm Producers		
Acinetobacter baumannii (n= 34)	22	Weak 10	Moderate 2	Strong -
Klebsiella pneumonia (n=8)	3	5	-	-
E. coli (n=2)	-	2	-	-
Serratia (n=2)	1	1	-	-

Out of total 46 bacterial isolates, 20 were biofilm producers, 2 isolates of Acinetobacter baumannii (A6, A43) were moderate biofilm producers and 18 isolates were weak biofilm producers including 10 strains of Acinetobacter baumannii (A5, A13, A16, A17, A18, A26, A35, A40, A41 & A42), 5 strains of Klebsiella pneumoniae (K8, K10, K14, K15 & K23), 2 strains of E. coli (E29 and E36) and 1 strain of Serratia (S30). Non-biofilm strains were A1, A2, A3, A4, A7, A9, K11, A12, A19, A20, A21, A22, A24, A25, A27, A28, A30, K31, A32, A33, S34, A37, A38, A44, A45 and A46. (Table 2).

DISCUSSION

Gram-negative bacilli are leading pathogens responsible for causing multiple infections like pneumonia, UTI, septicemia, meningitis and are also associated with hospital-acquired infections. These bacteria have developed resistance against numerous antibiotics, carbapenem resistance among these bacilli is a main interest around the globe. The present study was conducted to detect the formation of biofilm by Gram-negative bacilli which showed resistance to carbapenem. Out of 200 samples (blood, urine, pus, sputum, tracheal secretion and swab), 46 isolates were Gram-negative bacteria. Acinetobacter baumannii n=34 (74%) was prominent pathogen followed by Klebsiella pneumoniae n=8 (17%), E. coli n=2 (0.04%), and Serratia marcescens n=2 (0.04%). The result of our study was in accordance with the previously reported study conducted in Germany in which the similar trend of isolates was observed [12]. Brink and Adrian J. reported Acinetobacter baumannii, Enterobacteriaceae, and Pseudomonas aeruginosa among carbapenem resistance Gram-negative bacteria. [13]

In the present study, Acinetobacter baumannii (74%) was the most prevalent organism showing resistance to carbapenem. In another study Acinetobacter baumannii (67%) was also the most prevalent bacteria exhibiting resistance to carbapenem [14]. In another study performed by Di Wu and others in which they worked on Gram-negative bacilli that are resistant to carbapenem. According to their study the most prevalent carbapenem-resistant microorganism isolated was Acinetobacter baumannii 47/153 (33%) [15]. In our study other Gram-negative strains were Klebsiella pneumoniae (17%), E. coli (0.04%) and Serratia marcescens (0.04%). In another study conducted in China, the isolated Gram-negative bacilli resisting carbapenems are Klebsiella pneumoniae (n=333, 5.5%), E.coli (n=138, 1.0%) and Pseudomonas aeruginosa (317, 16.3%) and Acinetobacter baumannii (n=1001, 53.5%)[16]. In a study conducted in Algeria GNB which includes Acinetobacter baumannii (n=12), E. coli (n=12), P. aeruginosa (n=9), K. pneumoniae (n=20) were found to be resistant towards carbapenem. Acinetobacter (50%), being most resistant than Pseudomonas (22%), Klebsiella (20%), and E.coli (3.7%) (17) correlates with current study outcomes.

Carbapenems are regarded to be one of the last option antibiotics for the cure of deleterious diseases caused by Gram-negative bacteria. The constant rise and proliferation of resistance to these antibiotics is a chief public health problem [18]. In the present study, the Kirby-Bauer disk diffusion method was used to find the phenotypic resistance of the isolated bacteria against commonly used commercially available antibiotic disc. In our study, phenotypically most of the bacterial stains showed resistance towards carbapenem. In a study conducted by Paul G. Higgins and others, bacteria showed resistance to carbapenems more than 90% [19]. In a study performed in China, carbapenem resistance was found in accordance to our study which showed 152/154 (96%) samples resistant to carbapenem (meropenem and imipenem) [20]. According to a study in Brazil, carbapenem-resistance was found in 76.8% of A. baumannii isolates [21].

Furthermore, carbapenem-resistant strains (n=34) were analyzed for the presence of blaOXA-$_{23}$ gene. PCR was performed to find genotypic resistance in these strains. Of the total 34, genotypic resistance was found in 29 isolates. Acinetobacter baumannii 21/25 (84%) strains were most resistant to the OXA-23 gene. In our study, all E. coli 2/2 (100%) strains showed genotypic resistance towards $bla_{OXA\text{-}23}$, 3/5 (60%) strains of Klebsiella pneumoniae, and (1/2) strains of Serratia marcescens. In a study performed in China, 80.4% A. baumannii strains were for OXA-23 gene [22]. The prevalence of OXA-23 gene resistance was 100% in a study conducted in Qatar [23] and Nepal[24]. In a study from India, clinical isolates of E.coli n=14 (100%) showed the occurrence of OXA-23 gene[25]. The study conducted in Houston, Texas revealed that 11 out of 13 carbapenem-resistant bacterial isolates showed genes having acquired oxacillinases. Six bacterial isolates carried blaOXA-24, and 5 isolates possessed blaOXA-58. All isolates were

positive for blaOXA-51, the intrinsic gene characteristic of A. baumannii. A study conducted in 1995 to 2004 suggests that blaOXA-24 is a persistent problem in hospitals[26]. The study revealed that these oxacillinases (OXA-24, OXA-51 and OXA-58) cause carbapenem and multidrug resistance patterns among A. baumannii isolates which is in accordance with our findings.

In our study, Gram-negative strains were also differentiated on the basis of their ability to produce biofilm. The association between biofilm formation and antimicrobial resistance in Gram-negative isolates was statistically significant for carbapenems. According to the data available till date, this is the first time OXA-23 was found in Klebsiella pneumoniae in Pakistan and further study is required to isolate more resistant strains.

CONCLUSION

This research concluded that production of beta-lactamases and biofilm formation synergistically contributes to development of multi-drug resistance in Gram-negative bacteria. A higher trend of antibiotic resistance pattern was observed in isolated biofilm-forming carbapenem resistant bacteria. Acinetobacter baumannii was the most common infectious pathogen followed by Klebsiella pneumoniae, E. coli and Serratia marcescens and showed resistance against carbapenem drug. OXA-23, OXA-24, OXA-51 and OXA-58 genes have a significant role in resistance against carbapenem. Findings showed that 85% of Gram-negative bacteria were positive to OXA-23, 90% for OXA-24 gene, 83% for OXA-51 and 80% were positive to OXA-58. High prevalence of biofilm producing and multidrug-resistant bacteria foreshadows an impending concern worldwide.

Acknowledgement: The study was supported by Chughtai Lab, Lahore, University of Central Punjab, Lahore and Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan.

Conflict of Interest: None of the authors declare a conflict of interest.

REFERENCES

1. Ahmad I, Malak HA, Abulreesh HH. Environmental antimicrobial resistance and its drivers: A potential threat to public health. Journal of Global Antimicrobial Resistance. 2021 Dec 1;27:101-11.
2. Sharma D, Misba L, Khan AU. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrobial Resistance & Infection Control. 2019 Dec;8(1):1-0.
3. Sleiman A, Abou Fayad AG, Banna H, Matar GM. Prevalence and molecular epidemiology of carbapenem-resistant Gram-negative bacilli and their resistance determinants in the Eastern Mediterranean Region over the last decade. Journal of Global Antimicrobial Resistance. 2021 Jun 1;25:209-21.
4. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerging infectious diseases. 2011 Oct;17(10):1791.
5. Normark BH, Normark S. Evolution and spread of antibiotic resistance. Journal of internal medicine. 2002 Aug;252(2):91-106.
6. Wei Q, Ma LZ. Biofilm matrix and its regulation in Pseudomonas aeruginosa. International journal of molecular sciences. 2013 Oct 18;14(10):20983-1005.
7. Aparna MS, Yadav S. Biofilms: microbes and disease. Brazilian Journal of Infectious Diseases. 2008;12:526-30.
8. Odjadjare EE, Igbinosa EO, Mordi R, Igere B, Igeleke CL, Okoh AI. Prevalence of multiple antibiotics resistant (MAR) Pseudomonas species in the final effluents of three municipal wastewater treatment facilities in South Africa. International journal of environmental research and public health. 2012 Jun;9(6):2092-107.
9. Thi MT, Wibowo D, Rehm BH. Pseudomonas aeruginosa biofilms. International journal of molecular sciences. 2020 Nov 17;21(22):8671.
10. Vanegas JM, Parra OL, Jiménez JN. Molecular epidemiology of carbapenem resistant gram-negative bacilli from infected pediatric population in tertiary-care hospitals in Medellín, Colombia: an increasing problem. BMC infectious diseases. 2016 Dec;16(1):1-0.
11. Babar S, Haris M, Sikandar MZ, Siddiq UAB, Ahmad SJ, Shaheen F. Comparative Study of Static and Dynamic Hand Grip Endurance with Correlation of Deep Breathing among Pregnant Women; A Cross-sectional Study. PJMHS. 2023;17(3):220-3. https://doi.org/10.53350/pjmhs2023173220.
12. Siddiq UAB, Sikandar MZ, Ahmad SJ, Uppal MSK, Manzoor A, Tahir M. Recent Advances in the Management of Atrophic Tibial Non-Unions; An update using Extra Corporeal Shock Wave Therapy. PJMHS.2023;17(3):224–6. https://doi.org/10.53350/pjmhs2023173224
13. Khalid M, Sikandar MZ, Khan SZA, Khalid W, Tahir HMA, Tahir T. Frequency of Hyperbilirubinemia in Perforated Appendicitis - A Current Update on Diagnostics and Management. PJMHS. 2023;17(2):51-2. https://doi.org/10.53350/pjmhs202317251
14. Cheema UN, Zeb S, Irfan L, Sikandar MZ, Ashraf SA, Munir K. Impact of Topical v/s Systemic Steroids on Regaining Olfaction in Post Covid-19 Patients; A Randomized Controlled Trail. PJMHS. 2022;16(11):185–7. https://doi.org/10.53350/pjmhs20221611185
15. Uppal MSK, Bajwa AZ, Sikandar MZ, Siddiq UAB, Khan B, Bakar MA. Comparison of Outcomes of PHILOS for Proximal Humerus Fracture in Middle Aged Patients Based on Early Surgery vs Late Surgery. PJMHS. 2022;16(9):176-7. https://doi.org/10.53350/pjmhs22169176
16. Shah MR, Asif Z, Fatima A, Raheel N, Sikandar MZ. Knowledge, Attitudes and Practices Regarding Dengue Fever among Adult Population of Kurram Khyber Pakhtunkhwa. PJMHS. 2022;16(6):105-7. https://doi.org/10.53350/pjmhs22166105
17. Sikandar MZ, Bakar MA, Aleem H, Tufail A, Fatima A, Farooq, MU. Comparative Assessment of Trends of Social Anxiety and Social Avoidance during First and Second Wave of Covid-19. PJMHS. 2022;16(8):116-8. https://doi.org/10.53350/pjmhs22168116
18. Codjoe FS, Donkor ES. Carbapenem resistance: a review. Medical Sciences. 2017 Dec 21;6(1):1.
19. Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant Acinetobacter baumannii. Journal of Antimicrobial Chemotherapy. 2010 Feb 1;65(2):233-8.
20. Jiang M, Liu L, Ma Y, Zhang Z, Li N, Zhang F, Zhao S. Molecular epidemiology of multi-drug resistant Acinetobacter baumannii isolated in Shandong, China. Frontiers in Microbiology. 2016 Oct 21;7:1687.
21. Castilho SR, Godoy CS, Guilarde AO, Cardoso JL, André MC, Junqueira-Kipnis AP, Kipnis A. Acinetobacter baumannii strains isolated from patients in intensive care units in Goiânia, Brazil: Molecular and drug susceptibility profiles. PLoS One. 2017 May 5;12(5):e0176790.
22. Sun Y, Li M, Chen L, Chen H, Yu X, Ye J, Zhang Y, Ma C, Zhou T. Prevalence and molecular characterization of carbapenemase-producing Gram-negative bacteria from a university hospital in China. Infectious Diseases. 2016 Feb 1;48(2):138-46.
23. Olaitan AO, Morand S, Rolain JM. Emergence of colistin-resistant bacteria in humans without colistin usage: a new worry and cause for vigilance. International journal of antimicrobial agents. 2016 Jan 1;47(1):1-3.
24. Joshi PR, Acharya M, Kakshapati T, Lengtong Kam U, Thummeepak R, Sitthisak S. Co-existence of bla OXA-23 and bla NDM-1 genes of Acinetobacter baumannii isolated from Nepal: antimicrobial resistance and clinical significance. Antimicrobial Resistance & Infection Control. 2017 Dec;6(1):1-7.
25. Paul D, Ingti B, Bhattacharjee D, Maurya AP, Dhar D, Chakravarty A, Bhattacharjee A. An unusual occurrence of plasmid-mediated blaOXA-23 carbapenemase in clinical isolates of Escherichia coli from India. International journal of antimicrobial agents. 2017 May 1;49(5):642-5.
26. Castanheira M, Wanger A, Kruzel M, Deshpande LM, Jones RN. Emergence and clonal dissemination of OXA-24-and OXA-58-producing Acinetobacter baumannii strains in Houston, Texas: report from the SENTRY Antimicrobial Surveillance Program. Journal of clinical microbiology. 2008 Sep;46(9):3179-80.