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DETECTION OF RESISTANT GENES AMONG MDR *PSEUDOMONAS AERUGINOSA* INFECTION IN CHILDREN UNDERGOING CHEMOTHERAPY

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ABSTRACT

A Cross- sectional study was conducted for the detection of nosocomial resistant Beta-lactamase gene (*IMP, SPM, AIM* and *BIC*) against *Pseudomonas aeruginosa* in children undergoing chemotherapy. These genes play a significant role in multi drug resistance. *P. aeruginosa* is known to cause disease in immunocompromised patients e.g., cancer, HIV patients and burn patients. Also known as "superbug" due to resistant mechanism against the antibiotics. 10-15% of nosocomial infections are being caused by *P. aeruginosa* worldwide. About 300 samples (blood, sputum, urine & nasal) of children suffering from cancer were collected from a tertiary care hospital and prevalence of antibiotic resistance genes were analysed. Resistance against 13 antibiotics were tested in laboratory with disc diffusion method followed by PCR for the detection of genes. The results showed that the *P. aeruginosa* isolates were resistant against *ulfamethoxazole / Trimethoporim (SXT) 100%, Ciprofloxacin (CIP) 48%, and Ceftozane/Tezobactum (CT) 80%, Cefipime (FEP) 93%, Ceftazidime (CAZ) 40%, Amikacin (AK) 32%, Levofloxacin (LEV) 39% , Meropenem (MEM) 90%, Imipenem (IPM) 95%, Polymixin B (PB) 71%, Gentamicin (CN) 65%, Tobramycin (TOB) 28%, Tazobactum (TZP)* 24%.The PCR results showed that 91% isolates were positive for the *IMP* and 78% was positive for *AIM* and 68% was positive with *SPM* and 60% was positive with *BIC* genes. The prevalence of resistant genes *IMP, SPM, AIM, BIC* in *P. aeruginosa* is also on the increase in isolates from cancer patient. These findings are considered beneficial in understanding the mechanism involved in development of antibiotic resistance in *P. aeruginosa.*

Keywords: Cancer patients; drug resistance; antibiotics; AMR; antibiotic resistance

INTRODUCTION

Pseudomonas aeruginosa is one of the most important member of Genus *Pseudomonadaceae,* because it is involved in so many type of infections. *P. aeruginosa* appear as straight pink rods or marginally bent in shape under the Microscope. *Pseudomonas aeruginosa* are flagellated and are aerobic in nature [1]. These are gram negative bacteria and are also non-fermenting. Pseudomonas is famous because of its property of causing infections in patient which are already suffering from the diseases for example Patient suffering from various type of cancers, HIV infections and burn

patients. It is mostly involved in respiratory tract infections, Urinary tract infections, Gastro intestinal tract infections and bacteremia in humans. Its morbidity and mortality occur due to its infectious property [2]. The identification criteria of *Pseudomonas aeruginosa* in the laboratory is very simple as compared with other bacteria. Because it does not required any of the specific type of culture media and also specific conditions [3,4].

Multi drug resistant strains are responsible for hospital acquired infections, especially in the population at risk most commonly patient with cancer. MDR *P. aeruginosa* causes the major problems in cancer or cystic fibrosis patient [5,6]. Due to the MDR much cancer type develops resistant against the chemotherapy drugs, it is the most important factor in the failure of much type of chemotherapy drugs. It shows effect on a patient e.g., blood cancer, tumor, breast cancer lung and gastrointestinal tract cancer. Bacteremia due to the MDR bacteria is life threatening in to the cancer patient. The low permeability of its cell wall with mutation leading to the resistant via efflux pump, decreased level of porins plays a major role in development of problem in antibiotic therapy. Cancer also has the ability to develop resistance against the anti-cancer therapies. So, the prevalence of drug resistance cancer also is on the increase [7-10]. The awareness increases in the care of cancer patients. The patient of hematologic malignancies always on a risk to infection with gram negative bacteria e.g., MDR *P. aeruginosa* causes many type of infections neutropenia or lymphocyte dysfunction [11-13].

The Infections which are causes by *P. aeruginosa* infections are can be treated with antibiotic groups named as Carbapenems group; which includes *imipenem, meropenem*. In Renal failure patients the Aminoglycoside group of antibiotics are not recommended because of its nephrotoxicity [14,15]. Instead of aminoglycoside, Monobactam group of antibiotics especially aztreonam are generally reserved for serious infections caused by organism's resistant to other beta-lactam antibiotics. *P. aeruginosa* is well-known as an opportunistic pathogen. And it is linked with the several hospital acquired infections that are difficult to treat because the occurrence of resistance against multiple antibiotics.

MATERIALS AND METHODS

Sample Collection

This research was conducted at Faculty of Life sciences, UCP. About 300 samples were collected e.g., blood samples, sputum samples, urine samples and nasal samples from the patients which were cancer diagnosed, admitted in a tertiary care hospital in Lahore. The samples were appropriately labeled, transported to the research laboratory of the department of Microbiology, University of Central Punjab Lahore for further processing.

Isolation of *Pseudomonas*

After sample collection the samples were immediately transported to the laboratory. Then these samples were processed for further procedure of isolation and identification. The samples were inoculated on CLED Agar, MacConkey Agar and Blood agar. After labeling and inoculation these plates were placed in the incubator for 24 hours at 37^{o}C. Then After 24 hours Petri plates were checked for growth.

Identification of the Bacterial Isolates

The isolates were identified on colonial morphology, gram staining properties and biochemical tests.

Gram staining for microscopic identification

For microscopic identification of microbes gram staining was used. First step in the gram staining was to put the drop of normal saline on the slide and then the bacterial colony picked and made the smear on slide. The Slides were fixed with heating lamp followed by crystal violet stain for one minute, washing with distal water, Gram iodine staining for one minute and washed again with distal water. Finally slides were decolorized with acid alcohol and counter stained with safranin for about 45 seconds.

Biochemical characterization

For the identification of isolates, various biochemical tests were performed after gram staining. *Pseudomonas aeruginosa* Isolates shows the positive reactions for Citrate and Oxidase test while the Indole, Methyl Red, Voges-Proskauer, Lactose fermentation, Sucrose fermentation, Glucose fermentation, Urease and H2S reactions were negative.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility test was used for various antibiotics. The antibiotics used in this study were: *Sulfamethoxazole/Trimethoporim (SXT), Ciprofloxacin (CIP),* and *Ceftozane/Tezobactum (CT), Cefipime (FEP), Ceftazidime (CAZ), Amikacin (AK), Levofloxacin (LEV), Imipenem (IPM), Meropenem (MEM), Tazobactum (TZP), Polymixin B (PB), Gentamicin (CN),* and *Tobramycin (TOB).* Disk diffusion method was used for antibiotic susceptibility test.

Disk Diffusion Method (Kirby-Bauer Method)

Disk diffusion is commonly used method for antibiotic susceptibility test in clinical laboratories. In this method, Muller-Hinton agar was used with impregnated antibiotic disc placed on the agar. To prepare the medium 30 g of Muller-Hinton agar was added to one liter of distilled water in a flask and was stirred with magnetic stirrer.

The medium was than autoclaved in the autoclave at 121˚C for the period of 15-21 minutes. Autoclaved agar was cooled and poured into Petri plates for solidification of agar. Autoclaved Petri plates were used to avoid contamination.

Growth of colonies was streaked on the Petri plate with the help of cotton swab. The step was repeated to practice even distribution of the culture and set aside for 3 to 5 minutes. Antibiotics were placed on the inoculums afterwards by faintly pushing the disc with forceps to attached with inoculated organism. Discs used were *Sulfamethoxazole/Trimethoporim (SXT), Ciprofloxacin (CIP),* and *Ceftozane/Tezobactum (CT), Cefipime (FEP), Ceftazidime (CAZ), Amikacin (AK), Levofloxacin (LEV), Imipenem (IPM), Meropenem (MEM), Tazobactum (TZP), Polymixin B (PB), Gentamicin (CN),* and *Tobramycin (TOB).*The disks were placed at a distance of 24 mm to avoid overlapping of zones. After inoculation the disks were incubated the Petri plates for 24 hours at 37˚C. After incubation period the zone of inhibition was measured.

Methods for DNA extraction

For DNA extraction colonies of bacteria were used. Take one ml of distilled water in the test tube and add the colony of bacteria in it then boil for 10 minutes in the water bath. After boiling centrifuge the tube at 1000rpm for five minutes. For the purpose of PCR five micro litter of supernatant was used. In the second method for the DNA extraction microwave oven used to heat the bacterial colonies for 10 second followed by the centrifugation. Similarly, 5 µl of the supernatant was used for the PCR.

Table 1. Set of Primer used Amplification by PCR

Gene Names	Forward Primers	Reverse Primers
IMP	GAAGGCGTTTATGTTCATAC	GTATGTTTCAAGAGTGATGC
AIM	CTGAAGGTGTACGGAAACAC	GTTCGGCCACCTCGAATTG
SPM	AAAATCTGGGTACGCAAACG	ACATTATCCGCTGGAACAGG
BIC	TATGCAGCTCCTTTAAGGGC	TCATTGGCGGTGCCGTACAC

After DNA extraction by CTAB method, PCR performed for all of the isolates. The expected sizes of PCR products for the two sets of primers were 501 and 475 base-pairs (bp). For primers, the PCR mixture was incubated for 5 min at 95°C as initial denaturation, followed by initial DNA release and denaturation at 94˚C for 5 min, followed by 34 cycles of 94˚C for 30 s, 56˚C for 35 seconds and 72˚C for 1 minute, followed by a single, final, Extension step at 72°C for 5 min.

Gel Electrophoresis

2 % Agarose Gel is used to check the PCR products either the samples are positive for genes or they are negative.

2.0% Agarose

For the preparation of 2% gel, add 5.0 gram agarose was added in the 100 ml of 1X TBE buffer in a 600 ml beaker. After adding the agarose , heat was applied on beaker using a hot plate until the agarose was dissolved. Afterwards 7ul ethdiyam bromide was added in it and was poured in to the Gel tray. After solidification the samples were loaded in to the wells of solid gel. After loading the samples electric field was applied at 120 volt for 30 min.

RESULTS

Sample Collection

Total 300 samples collected from Cancer patients, admitted different wards of for this research. 300 samples include 100 blood samples, 100 sputum sample, 50 urine samples and 50 nasal samples shown in Fig 1.

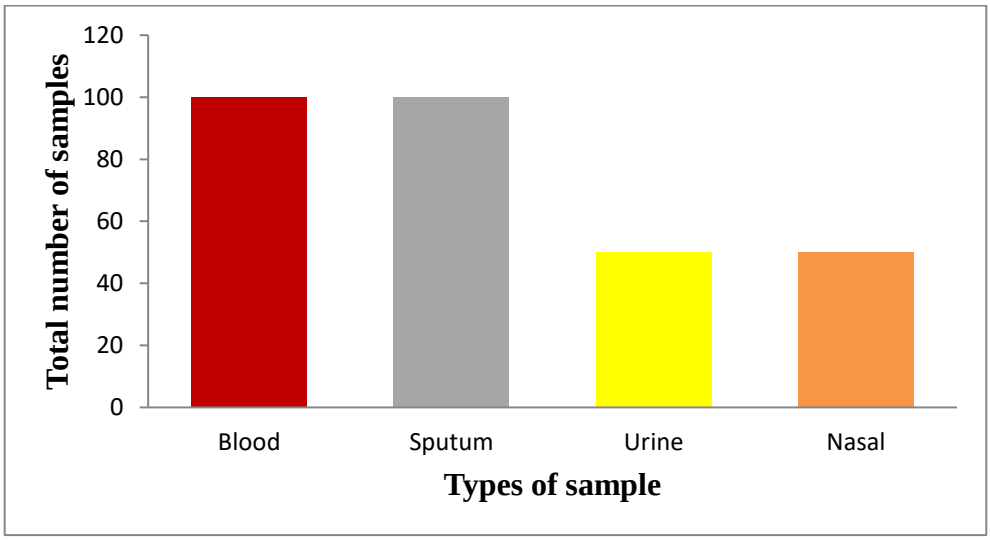

Figure 1. Describes sample collection.

On the basis of various laboratory tests of Blood, Sputum, urine and nasal it was noted that all of the samples were positive for different pathogens. Prevalence of pathogen is shown in Fig 2.

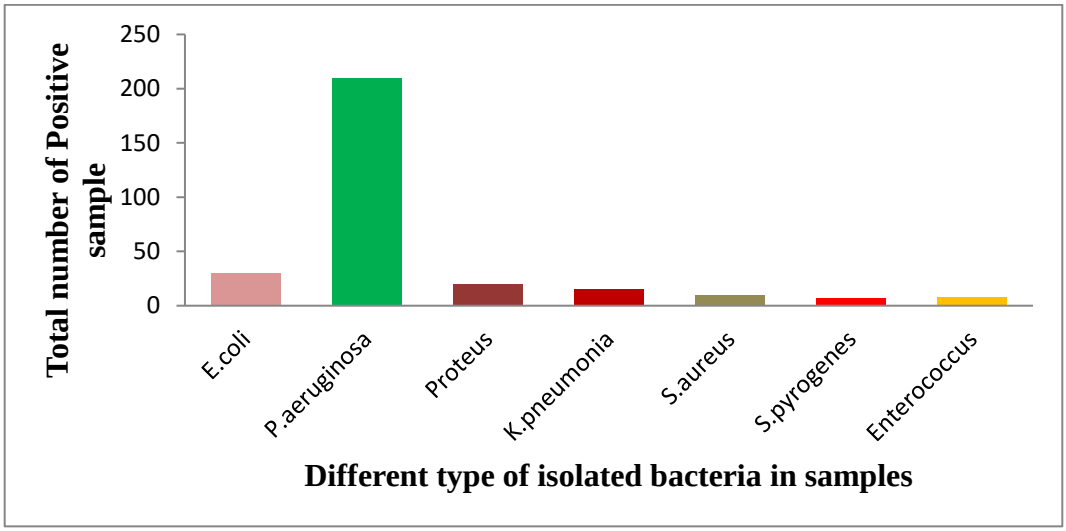

Figure 2: It describes the Prevalence of different bacteria in isolated samples

Out of these 300 samples we separated the 225 positive samples of *P. aeruginosa as* shown in Fig 3.

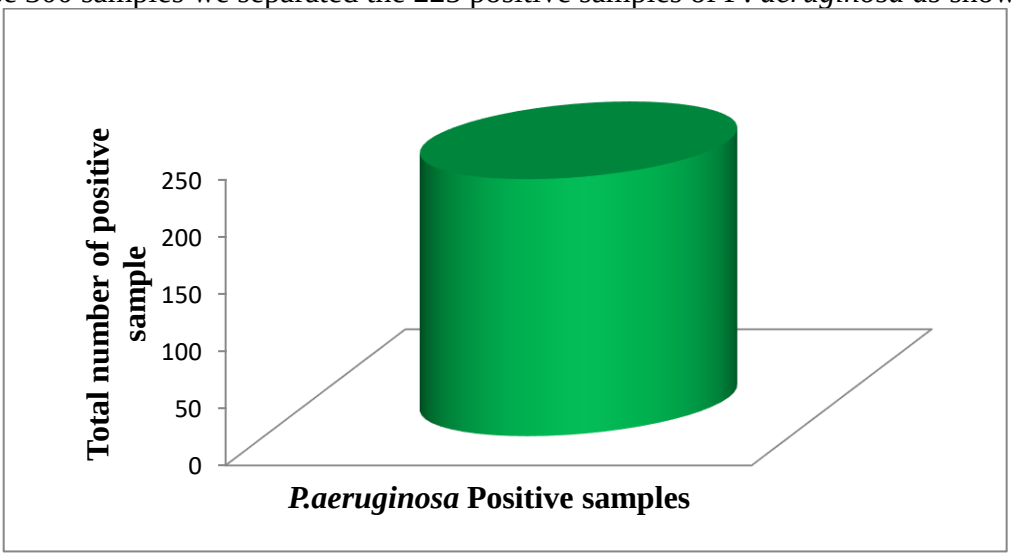

Figure 3: Total number of positive *Pseudomonas aeruginosa* samples.

And out of these 300 samples 87 blood samples are positive with *P. aeruginosa*, 60 sputum samples or 40 urine samples are positive with *P. aeruginosa* and 38 nasal samples are positive with *P. aeruginosa* shown in Fig 4.

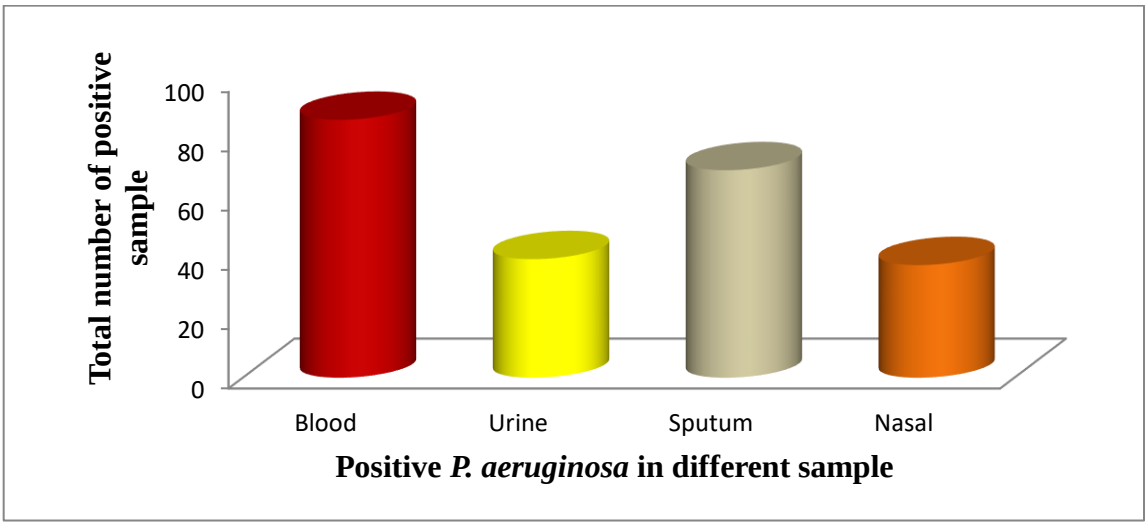

Figure 4: *P. aeruginosa* in different samples

Prevalence of *Pseudomonas aeruginosa* on basis of Cancer type

Prevalence of *P. aeruginosa* on the basis of Cancer type shows different types, as shown in Table 2. MDR *P. aeruginosa* shows prevalence in all type of cancer in children's undergoing chemotherapy

Table 2. *P. aeruginosa* prevalence in different type of cancer in children undergoing through chemotherapy.

	Positive Sample	**Prevalence**
Acute leukemia (ALL)	75	33%
Chronic leukemia (CLL)	55	24%
Acute Myeloid leukemia (AML)	46	18%
Osteo Sarcoma (OS)	15	0.06%
Ewing's sarcoma	07	0.03%
Hodgkin's lymphoma	17	0.07%
Non Hodgkin's lymphoma	10	0.04%

Isolation and Identification

P. aeruginosa grown on blood agar and MacConkey agar showing β hemolytic colonies which are surrounded by bluish green coloration. And Pale yellowish non lactose fermenters colonies on Blood agar. *Pseudomonas aeruginosa* is able to grow at temperatures as high as 42˚C.

Gram staining

P. aeruginosa are gram negative rods, shows pink color rods under microscope after gram staining *Pseudomonas aeruginosa* are flagellated and motile. Gram negative rods in pink color.

Biochemical profiling for identification

Various Biochemical tests performed for the confirmation and identification of *P. Aerugenosa* isolates.

Antimicrobial Susceptibility Testing (AST)

This test was used for various antibiotics. The antibiotics used in this study were: Sulfamethoxazole/Trimethoporim (SXT), Ciprofloxacin (CIP), and Ceftozane/Tezobactum (CT), Cefipime (FEP), Ceftazidime (CAZ), Amikacin (AK), Levofloxacin (LEV), Imipenem (IPM), Meropenem (MEM), Tazobactum (TZP), Polymixin B (PB), Gentamicin (CN), and Tobramycin (TOB). Disk diffusion method was used for antibiotic susceptibility test. In this test Pseudomonas aeruginosa shows antimicrobial drugs resistant against Sulfamethoxazole/Trimethoporim (SXT) 100%, Ciprofloxacin (CIP) 48%, and Ceftozane/Tezobactum (CT) 80%, Cefipime (FEP) 93%, Ceftazidime (CAZ) 40%, Amikacin (AK) 32%, Levofloxacin (LEV) 39%, Meropenem (MEM) 90%, Imipenem (IPM) 95%, Polymixin B (PB) 71%, Gentamicin (CN) 65%, Tobramycin (TOB) 28%, Tazobactum (TZP) 24% as shown in Table 3.

Table 3. Prevalence of Antibiotic Resistance

Antibiotic	Code	Resistant Sample	Sensitive Sample	Prevalence of Resistance
Sulfamethoxazole	SXT	225	00	100%
Ciprofloxacin	CIP	110	115	48%
Ceftozane	CT	180	45	80%
Cefipime	FEP	210	15	93%
Ceftazidime	CAZ	90	135	40%
Amikacin	AK	73	152	32%
Levofloxacin	LEV	88	137	39%
Meropenem	MEM	202	23	90%
Imipenem	IMP	214	11	95%
Polymixin B	PB	160	65	71%
Gentamicin	CN	148	77	65%
Tobramycin	TOB	64	161	28%
Tazobactum	TZP	55	180	24%

Detection of *IMP* Resistant Gene

IMP gene is detected in *Pseudomonas aeruginosa* from cancer patient which were undergoing chemotherapy. It is detected from 205 samples.

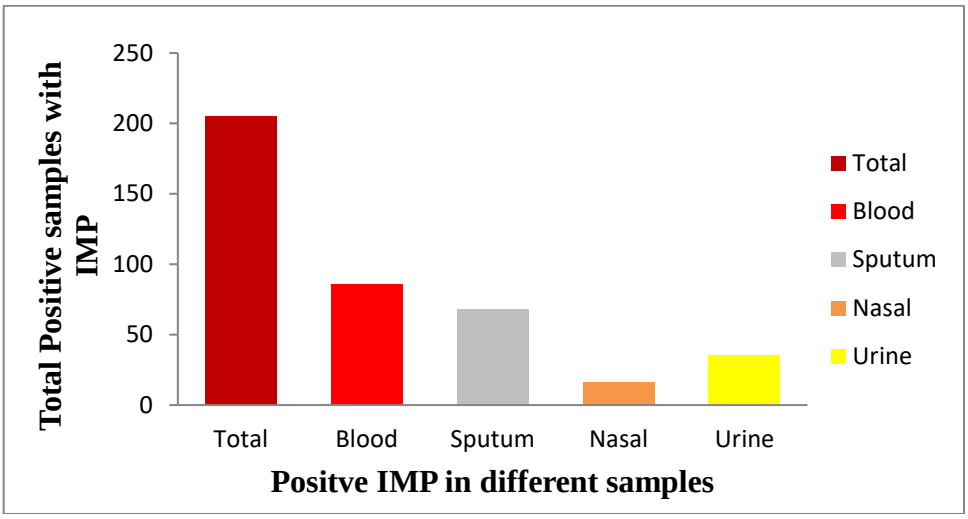

Figure 5. Describes the detection of IMP genes in different samples.

Detection of *SPM* Resistant Gene

SPM gene was detected in *Pseudomonas aeruginosa* from cancer patient which is undergoing from chemotherapy. It is detected from 153 samples.

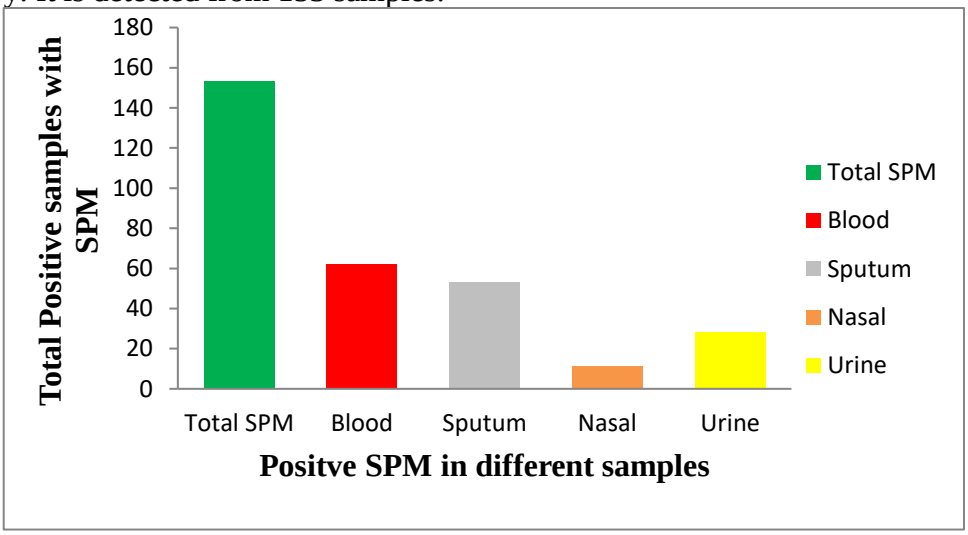

Figure 6. Detection of SPM genes

Detection of *AIM* Resistant Gene

AIM gene is detected in *Pseudomonas aeruginosa* from cancer patient which is undergoing from chemotherapy. It is detected from 177 samples.

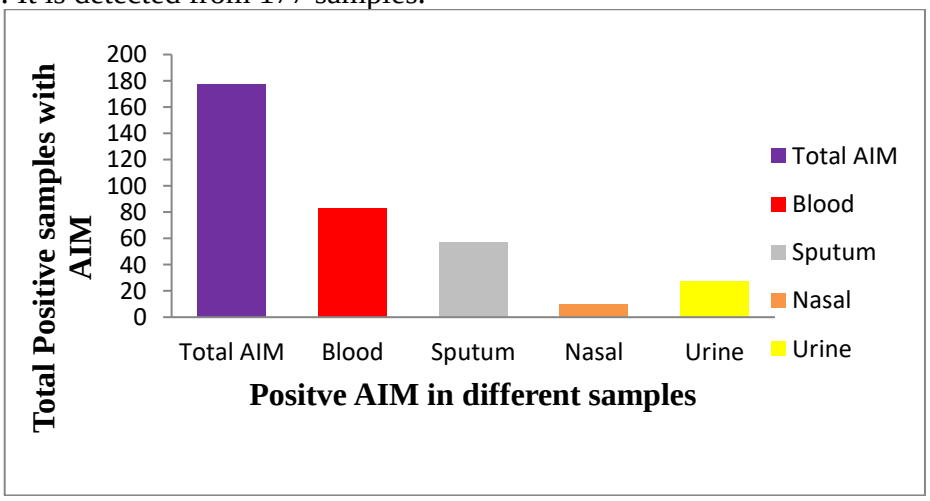

Figure 7. Describes the detection of AIM genes in different samples

Detection of *BIC* Resistant Gene

BIC gene is detected in *Pseudomonas aeruginosa* from cancer patient which is undergoing from chemotherapy. It is detected from 135.

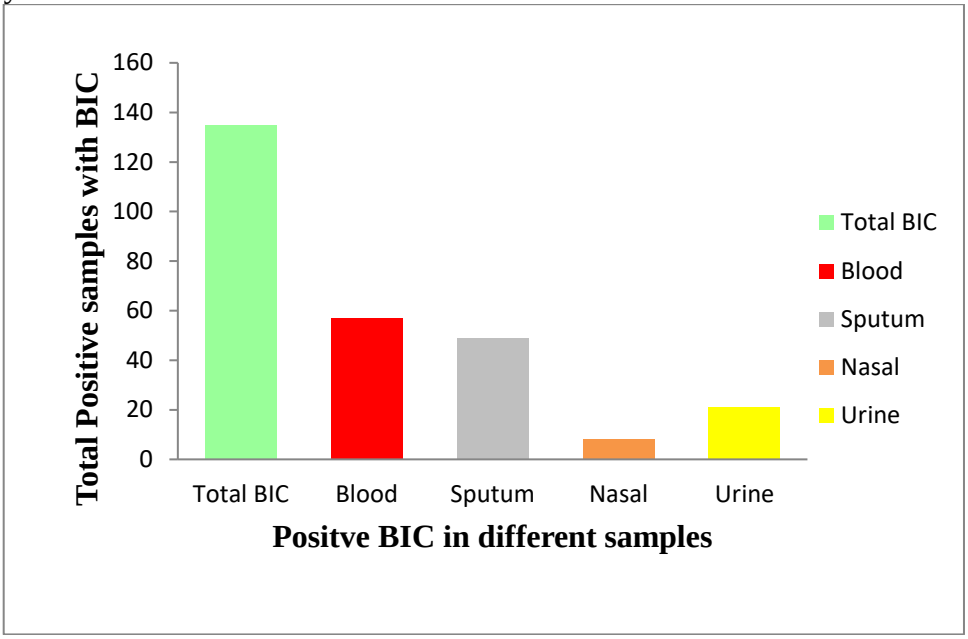

Figure 8. Describe the Detection of BIC genes in different samples.

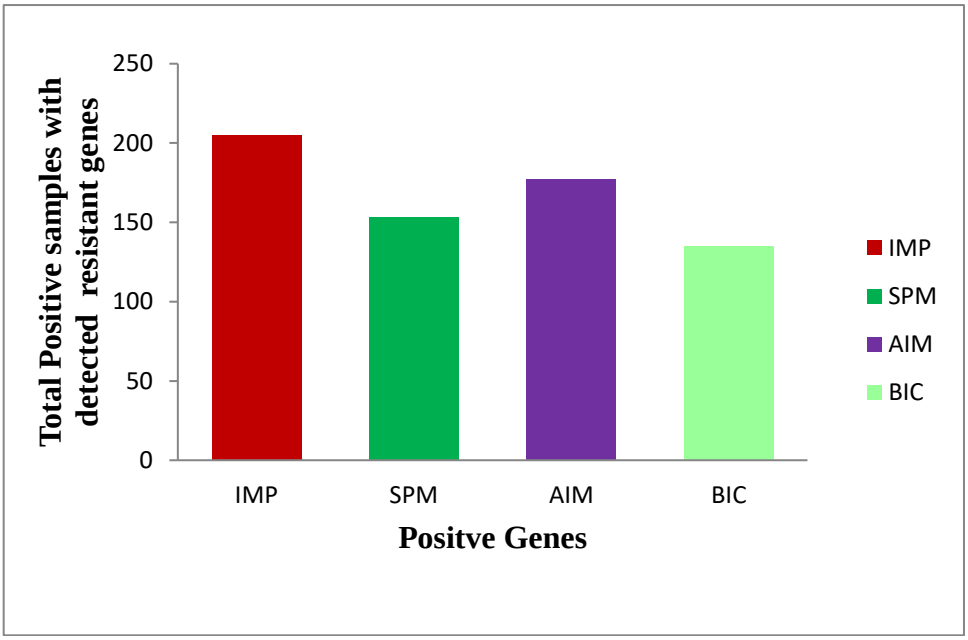

Figure 9. Total positive samples of *P. aeruginosa with* resistant genes

DISCUSSION

Pseudomonas aeruginosa are non-fermenters and Gram-negative bacteria. Now a day's *P. aeruginosa* is very famous for role in hospital-acquired infections. Due to this infectious property of *P. aeruginosa* mortality and morbidity occurs [16]. In Hospitals and specifically in intensive care units *P. aeruginosa* is most general pathogen, which causes the diseases in immune-compromised patients and sometimes causes death. Since the entry of antibiotics in clinical treatment, *P. aeruginosa* is known as a superbug. *P. aeruginosa* has been develop increasingly more complicated resistant mechanism against the antibiotics. *P. aeruginosa* causes so many infections in humans because of the resistance nature against all available antibiotics, and has become a super bug [17-19].

Multi drug resistance strains responsible for hospital acquired diseases, especially in the population at risk most commonly patient with cancer. MDR *P. aeruginosa* cause the major problems in cancer or cystic fibrosis patient [20-22]. Due to multi drug resistance many cancer type develops resistant against the chemotherapy drugs, it is the most important factor in the failure of many type of chemotherapy drugs. It shows effect on a patient e.g. blood cancer, tumor, breast cancer lung and gastrointestinal tract cancer. Bacteremia due to the MDR *P. aeruginosa* is life threatening into the cancer patient. The low permeability of its cell wall with mutation leading to the resistant via efflux pump, decreased level of porins plays a major role in development of problem in antibiotic therapy. So due to this reason MDR *P. aeruginosa* is concerning [23-25].

Cancer also have the ability to develop resistance against the anti-cancer therapies. So the prevalence of drug resistance cancer also on the increase. Now days the infection caused by MDR *P. aeruginosa* is infections are difficult to treat in immune-compromised patients, because of this reason mortality occurs. The Plasmid and Transposons are responsible for the transport of resistant gene between cells [26-29]. Due to this high infection rate, *P. aeruginosa* are most importantly involved in critically ill patients, for example Such as the patients which are suffering from leukemia and cancer. The MDR *P. aeruginosa* causes mortality in these patients [30].

The aim of this study was to isolate the MDR *P. aeruginosa* and to detect the resistant gene from clinical samples of cancer patients. Fifty positive samples were collected. The prevalence of *P. aeruginosa* in this study was 33%, which shows that *P. aeruginosa* may potentially be significant for causing infections in cancer patient. The highest prevalence of *P. aeruginosa* found in blood sample was 50% in comparison with urine sample which was 20% and the prevalence of nasal sample was 30% and the high prevalence relates to the results found in a study by [31-34]. Cancer is a problem in a medical field because due to the low immunity and compromised host defense microorganism has an idol condition to cause the disease. Multi drug resistance strains responsible for hospital acquired diseases, especially in the population at risk most commonly patient with cancer. MDR *P. aeruginosa* cause the major problems in cancer or cystic fibrosis patient [35]. The prevalence of *P. aeruginosa* in blood sample indicates that it possess a high risk in causing bacteremia in cancer patient. The intermediate prevalence of *P. aeruginosa* in nasal sample indicates that it may be transmitted by the physical contact and due to which bacteria may be easily transmitted. The patients with low immunity and with compromised host defense at high risk of infection. The cross contamination with the hospital isolates play a major role in causing nosocomial infections [23,36,37]. The prevalence of Pseudomonas in men was 34% in women was 46% and in child was 20%. Research reveals that the prevalence of this organism in males was found higher than in females.

The best antibiotics, which can be used against the infections of multi drug resistant gram-negative bacteria are carbapenems. In current years, the countries which are seriously facing the problem of antibiotic resistance, Egypt is one of them [38]. In the present research resistance is high against all the commercially available antibiotics among *P. aeruginosa* isolated from the Shoukat Khanam Memorial Hospital and research center. The prevalence of resistance against Meropenem is 68% and the resistance against the Imipenem is 92%. The high rate of carbapenem resistance indicates the less treatment option in Hospital. This is due to the increase in antibiotic usage in the last past years due to this, the bacteria modify the mechanism of resistance. In many other developing countries the situation is same the resistance is on the increase. Among gram negative bacteria the *P. aeruginosa* and *Acenitobacter* shows the high level of resistance against the Imipenem which is 37.03% the study was conducted by [23,39]. Mahmoud *et al.,* 2013 conducted a research on a *P. aeruginosa* and reported that the 33.3% isolate resistant to Imipenem. In the countries which are located in a middle east Imipenem resistance is on the increase. In the Saudi Arabia the resistance against the Imipenem is 38.57% reported in 2011 [26]. According to the European surveillance system in six different European countries the carbapenem resistance is reported about 25%. The highest resistance against the carbapenem reported in Greece which was 51% [19]. The Bacteria has different type of enzyme which plays an important role in the resistance.

The most commonly reported families are *IMP* which was firstly isolated in Japan. The *VIM* family was firstly isolated from Italy. *SPM* and *AIM* which was firstly isolated from Brazil. *IMP* and *VIM* producing *P. aeruginosa* are reported worldwide in different areas. In this current research *IMP* was the most commonly detectable gene among *P. aeruginosa.* The prevalence of *IMP* gene among *P. aeruginosa* was 91%. In the 78% isolates the *AIM* gene is detected. The result of previous studies supports the findings of our research. Previous studies demonstrate that the *VIM* is the most commonly prevalent gene among the *P. aeruginosa* and *IMP* gene prevalence also very high the greatest clinical threat. In all over the world *IMP* gene is associated with the hospital outbreaks due to the MBL producing *P. aeruginosa* [27,31,40]. In our study, The PCR results showed that 91% isolates were positive for the *IMP* and 78% was positive for *AIM* and 68% was positive with *SPM* and 60% was positive with *BIC* genes.

CONCLUSION

In this current research the high resistance reported in multi drug resistance P. aeruginosa in cancer patients. The prevalence of resistant genes *IMP, SPM, AIM, BIC* in *P. aeruginosa* are also on the increase in isolates from cancer patients. These findings are considered beneficial in understanding the mechanism involved in development of antibiotic resistance in *P. aeruginosa*.

REFERENCES

1. Ahmed, N.; Ali, Z.; Riaz, M.; Zeshan, B.; Wattoo, J.I.; Aslam, M.N. Evaluation of antibiotic resistance and virulence genes among clinical isolates of Pseudomonas aeruginosa from cancer patients. *Asian Pacific journal of cancer prevention: APJCP* **2020**, *21*, 1333.
2. Ahmed, N.; Habib, S.; Muzzammil, M.; Rabaan, A.A.; Turkistani, S.A.; Garout, M.; Halwani, M.A.; Aljeldah, M.; Al Shammari, B.R.; Sabour, A.A. Prevalence of Bacterial Pathogens among Symptomatic–SARS-CoV-2 PCR-Negative Patients. *Microorganisms* **2022**, *10*, 1978.
3. Rizvi, A.; Saeed, M.U.; Nadeem, A.; Yaqoob, A.; Rabaan, A.A.; Bakhrebah, M.A.; Al Mutair, A.; Alhumaid, S.; Aljeldah, M.; Al Shammari, B.R. Evaluation of Bi-Lateral Co-Infections and Antibiotic Resistance Rates among COVID-19 Patients in Lahore, Pakistan. *Medicina* **2022**, *58*, 904.
4. Zeb, S.; Mushtaq, M.; Ahmad, M.; Saleem, W.; Rabaan, A.A.; Naqvi, B.S.Z.; Garout, M.; Aljeldah, M.; Al Shammari, B.R.; Al Faraj, N.J. Self-Medication as an Important Risk Factor for Antibiotic Resistance: A Multi-Institutional Survey among Students. *Antibiotics* **2022**, *11*, 842.
5. Ahmed, N.; Khan, M.; Saleem, W.; Karobari, M.I.; Mohamed, R.N.; Heboyan, A.; Rabaan, A.A.; Mutair, A.A.; Alhumaid, S.; Alsadiq, S.A. Evaluation of bi-lateral co-infections and antibiotic resistance rates among COVID-19 patients. *Antibiotics* **2022**, *11*, 276.
6. Ahmed, N.; Zeshan, B.; Naveed, M.; Afzal, M.; Mohamed, M. Antibiotic resistance profile in relation to virulence genes fimH, hlyA and usp of uropathogenic E. coli isolates in Lahore, Pakistan. *Trop. Biomed* **2019**, *36*, 559-568.
7. Ahmed, N.; Abusalah, M.A.H.A.; Farzand, A.; Absar, M.; Yusof, N.Y.; Rabaan, A.A.; AlSaihati, H.; Alshengeti, A.; Alwarthan, S.; Alsuwailem, H.S. Updates on Epstein–Barr Virus (EBV)-Associated Nasopharyngeal Carcinoma: Emphasis on the Latent Gene Products of EBV. *Medicina* **2022**, *59*, 2.
8. Al-Mhanna, S.B.; Ghazali, W.S.W.; Mohamed, M.; Rabaan, A.A.; Santali, E.Y.; Alestad, J.H.; Santali, E.Y.; Arshad, S.; Ahmed, N.; Afolabi, H.A. Effectiveness of physical activity on immunity markers and quality of life in cancer patient: a systematic review. *PeerJ* **2022**, *10*, e13664.
9. Mohd Salim, N.H.; Mussa, A.; Ahmed, N.; Ahmad, S.; Yean Yean, C.; Hassan, R.; Uskoković, V.; Mohamud, R.; Che Jalil, N.A. The Immunosuppressive Effect of TNFR2 Expression in the Colorectal Cancer Microenvironment. *Biomedicines* **2023**, *11*, 173.

10. Mussa, A.; Mohd Idris, R.A.; Ahmed, N.; Ahmad, S.; Murtadha, A.H.; Tengku Din, T.A.D.A.A.; Yean, C.Y.; Wan Abdul Rahman, W.F.; Mat Lazim, N.; Uskoković, V. High-dose vitamin C for cancer therapy. *Pharmaceuticals* **2022**, *15*, 711.
11. Parveen, S.; Saqib, S.; Ahmed, A.; Shahzad, A.; Ahmed, N. Prevalence of MRSA colonization among healthcare-workers and effectiveness of decolonization regimen in ICU of a Tertiary care Hospital, Lahore, Pakistan. *Advancements in Life Sciences* **2020**, *8*, 38-41.
12. Rasool, Z.; Noreen, H.; Anjum, A.; Rizvi, A.; Rabaan, A.A.; Halwani, M.A.; Sabour, A.A.; Aljeldah, M.; Shammari, B.R.A.; Alhajri, S.M. Genotypic and Phenotypic Characterization of Erythromycin-Resistant Staphylococcus aureus Isolated from Bovine Mastitis and Humans in Close Contact. *Tropical Medicine and Infectious Disease* **2022**, *8*, 26.
13. Sohail, M.; Muzzammil, M.; Ahmad, M.; Rehman, S.; Garout, M.; Khojah, T.M.; Al-Eisa, K.M.; Breagesh, S.A.; Hamdan, R.M.A.; Alibrahim, H.I. Molecular Characterization of Community-and Hospital-Acquired Methicillin-Resistant Staphylococcus aureus Isolates during COVID-19 Pandemic. *Antibiotics* **2023**, *12*, 157.
14. Mustafai, M.M.; Hafeez, M.; Munawar, S.; Basha, S.; Rabaan, A.A.; Halwani, M.A.; Alawfi, A.; Alshengeti, A.; Najim, M.A.; Alwarthan, S. Prevalence of Carbapenemase and Extended-Spectrum β-Lactamase Producing Enterobacteriaceae: A Cross-Sectional Study. *Antibiotics* **2023**, *12*, 148.
15. Rabaan, A.A.; Eljaaly, K.; Alhumaid, S.; Albayat, H.; Al-Adsani, W.; Sabour, A.A.; Alshiekheid, M.A.; Al-Jishi, J.M.; Khamis, F.; Alwarthan, S. An Overview on Phenotypic and Genotypic Characterisation of Carbapenem-Resistant Enterobacterales. *Medicina* **2022**, *58*, 1675.
16. Vakulenko, S.B.; Mobashery, S. Versatility of aminoglycosides and prospects for their future. *Clinical microbiology reviews* **2003**, *16*, 430-450.
17. Struelens, M.; Monnet, D.; Magiorakos, A.; O'Connor, F.S.; Giesecke, J. New Delhi metallo-beta-lactamase 1-producing Enterobacteriaceae: emergence and response in Europe. *Eurosurveillance* **2010**.
18. Sutcliffe, J.; Grebe, T.; Tait-Kamradt, A.; Wondrack, L. Detection of erythromycin-resistant determinants by PCR. *Antimicrobial agents and chemotherapy* **1996**, *40*, 2562-2566.
19. Tam, V.H.; Chang, K.-T.; Abdelraouf, K.; Brioso, C.G.; Ameka, M.; McCaskey, L.A.; Weston, J.S.; Caeiro, J.-P.; Garey, K.W. Prevalence, resistance mechanisms, and susceptibility of multidrug-resistant bloodstream isolates of Pseudomonas aeruginosa. *Antimicrobial agents and chemotherapy* **2010**, *54*, 1160-1164.
20. Poirel, L.; Revathi, G.; Bernabeu, S.; Nordmann, P. Detection of NDM-1-producing Klebsiella pneumoniae in Kenya. *Antimicrobial agents and chemotherapy* **2011**, *55*, 934-936.
21. Rubin, J.; Victor, L.Y. Malignant external otitis: insights into pathogenesis, clinical manifestations, diagnosis, and therapy. *The American journal of medicine* **1988**, *85*, 391-398.
22. Silby, M.W.; Winstanley, C.; Godfrey, S.A.; Levy, S.B.; Jackson, R.W. Pseudomonas genomes: diverse and adaptable. *FEMS microbiology reviews* **2011**, *35*, 652-680.
23. Mittal, R.; Aggarwal, S.; Sharma, S.; Chhibber, S.; Harjai, K. Urinary tract infections caused by Pseudomonas aeruginosa: a minireview. *Journal of infection and public health* **2009**, *2*, 101-111.
24. Nixon, G.M.; Armstrong, D.S.; Carzino, R.; Carlin, J.B.; Olinsky, A.; Robertson, C.F.; Grimwood, K. Clinical outcome after early Pseudomonas aeruginosa infection in cystic fibrosis. *The Journal of pediatrics* **2001**, *138*, 699-704.
25. Poirel, L.; Lambert, T.; Türkoglü, S.; Ronco, E.; Gaillard, J.-L.; Nordmann, P. Characterization of Class 1 Integrons from Pseudomonas aeruginosa That Contain the bla VIM-2Carbapenem-Hydrolyzing β-Lactamase Gene and of Two Novel Aminoglycoside Resistance Gene Cassettes. *Antimicrobial agents and chemotherapy* **2001**, *45*, 546-552.
26. Pizarro-Cerdá, J.; Cossart, P. Bacterial adhesion and entry into host cells. *Cell* **2006**, *124*, 715-727.

27. Rátkai, C. Characterization of medically important Pseudomonas aeruginosa isolates. szte, 2011.
28. Rodríguez-Martínez, J.-M.; Poirel, L.; Nordmann, P. Molecular epidemiology and mechanisms of carbapenem resistance in Pseudomonas aeruginosa. *Antimicrobial agents and chemotherapy* **2009**, *53*, 4783-4788.
29. Shakil, S.; Khan, R.; Zarrilli, R.; Khan, A.U. Aminoglycosides versus bacteria–a description of the action, resistance mechanism, and nosocomial battleground. *Journal of biomedical science* **2008**, *15*, 5-14.
30. Rossolini, G.; Mantengoli, E. Treatment and control of severe infections caused by multiresistant Pseudomonas aeruginosa. *Clinical Microbiology and Infection* **2005**, *11*, 17-32.
31. Lister, P.D.; Wolter, D.J.; Hanson, N.D. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clinical microbiology reviews* **2009**, *22*, 582-610.
32. Livermore, D.M. beta-Lactamases in laboratory and clinical resistance. *Clinical microbiology reviews* **1995**, *8*, 557-584.
33. Meenakumari, S.; Verma, S.; Absar, A.; Chaudhary, A. Antimicrobial susceptibility pattern of clinical isolates of Pseudomonas aeruginosa in an Indian cardiac hospital. *Int J Engineering Sci Tech* **2011**, *3*, 7117-7124.
34. Mesaros, N.; Glupczynski, Y.; Avrain, L.; Caceres, N.E.; Tulkens, P.M.; Van Bambeke, F. A combined phenotypic and genotypic method for the detection of Mex efflux pumps in Pseudomonas aeruginosa. *Journal of antimicrobial chemotherapy* **2007**, *59*, 378-386.
35. Maki, D.G.; Tambyah, P.A. Engineering out the risk for infection with urinary catheters. *Emerging infectious diseases* **2001**, *7*, 342.
36. Janice, Y. Laboratory diagnosis of NDM-1 and other carbapenem-resistant Enterobacteriaceae. *Medical Bulletin* **2011**, *16*.
37. Navon-Venezia, S.; Leavitt, A.; Schwaber, M.J.; Rasheed, J.K.; Srinivasan, A.; Patel, J.B.; Carmeli, Y.; Group, I.K.K.S. First report on a hyperepidemic clone of KPC-3-producing Klebsiella pneumoniae in Israel genetically related to a strain causing outbreaks in the United States. *Antimicrobial agents and chemotherapy* **2009**, *53*, 818-820.
38. Poirel, L.; Héritier, C.; Tolün, V.; Nordmann, P. Emergence of oxacillinase-mediated resistance to imipenem in Klebsiella pneumoniae. *Antimicrobial agents and chemotherapy* **2004**, *48*, 15-22.
39. Noyal, M.; Menezes, G.; Harish, B.; Sujatha, S.; Parija, S. Simple screening tests for detection of carbapenemases in clinical isolates of nonfermentative Gram-negative bacteria. *Indian Journal of Medical Research* **2009**, *129*, 707.
40. Ranjan, K.P.; Ranjan, N.; Bansal, S.K.; Arora, D. Prevalence of Pseudomonas aeruginosa in post-operative wound infection in a referral hospital in Haryana, India. *Journal of laboratory physicians* **2010**, *2*, 74.