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Antibiotic susceptibility and molecular detection of *MEXT* gene of *Pseudomonas aeruginosa* isolated from burned patients

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Abstract

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic human pathogen and a leading cause of disease and death in burned patients. Nosocomial infections caused by *P. aeruginosa* are increasing worldwide that can be attributed to uncontrolled use of antibiotics in hospitals and community. The present study was aimed to decipher the antibiotic susceptibility and molecular detection of *MEXT* gene in multidrug resistant *P. aeruginosa* isolates from burned patients. A total of 100 swab samples from burned patients were collected and cultured on cetrimide agar plates followed by enrichment in nutrient broth. Bio characterization was done for positive pigment producing isolates. Antibiotic susceptibility was assessed by Kirby Bauer disk diffusion method to commonly used antibiotics; amikacin, tobramycin, ciprofloxacin, colistin, carbenicillin, meropenem, and ceftazidim. The *MEXT* gene was amplified in multidrug resistant (MDR) isolates. It was found that 44% isolates were positive for *P. aeruginosa* giving pigment production and positive citrate and oxidase tests. Antibiogram results revealed that 56.8% isolates (13/44) were multidrug resistant. The *MEXT* gene in selected MDR isolates was detected with 216bp size. In conclusion, colistin and meropenem could be effective in treating *P. aeruginosa* infections and *MEXT* gene modulates the induction of multidrug efflux system that further modulates antibiotic resistance to diverse range of antibiotics in *P. aeruginosa*.

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Introduction

Nosocomial infections are becoming a major problem worldwide with 63.8% infection rate. It has been reported that almost 2.5 million people in United States had burn infection each year. Due to burn wound infection, natural cutaneous barrier is lost which permit more microbial colonization making the infection more prolong and panic (Mayhall, 2003).In burn infections, many types of organisms grow and among them *Pseudomonas aeruginosa (P. aeruginosa)* is most common that contributes 11-13.8% of the infections with incidence rate reported to be 13.2-22.6% (Drenkard, 2003). *P. aeruginosa* is a Gram negative bacterium inhabiting moist soil, marshes and coastal marine habitats. It also has the potential to grow on living animal and plant tissues. The biofilm forming ability enables it to grow on moist rocks, soil surfaces and on moist surface of catheters (Stover *et al.,* 2000).

During last centuries, *P. aeruginosa* has been reported a major opportunistic pathogen of humans due to resistance to many common antibiotics and disinfectants (Waszczuk *et al.,* 2012). It has many mechanisms of antimicrobial resistance including multidrug efflux pump, β-lactamases, and down regulation of outer membrane porins. The high prevalence is due to the induction of different efflux pumps that mediates antibiotic resistance to diverse range of antibiotics (Schweizer, 2003).The emergence of multidrug resistant (MDR) strain of *P. aeruginosa* has created a problematic issue among burnt patients and has created a challenge in treatment of nosocomial infections. This MDR *P. aeruginosa* is becoming resistance to commonly used antibiotics like colistin, meropinem, tigecycline and doripenem (Richard *et al.,* 1994). The ability to acquire multidrug resistance is due to the presence of different efflux systems. The MexEF-OprN is one of these multi-drug efflux systems involved in antibiotic resistance to a variety of antibiotics. The gene called *MEXT* belonging to LysR family mediates the induction of this system making *P. aeruginosa* resistant to diverse range of antibiotics (Maseda *et al.,* 2000).The objective of this study was to check the antibiotic susceptibility and molecular detection of *MEXT* gene from multidrug resistant *P. aeruginosa* isolated from burned patients.

Materials and methods

The following study was conducted at Institute of Microbiology, University of Agriculture Faisalabad, Pakistan. Before collection of samples, an informed consent of patients was taken and they were told about the purpose of sample collection and research study. A total of 100 swab samples from skin of the burned patients were collected from Burn Unit of Allied hospital, Punjab Medical University, Faisalabad. Collected samples were enriched in nutrient broth and plated on cetrimide agar plates following incubation at 37°C for 24 hours. After overnight incubation, presumptive selection of *P. aeruginosa* isolates was done macroscopically on the basis of colony morphology and pigment production and microscopically through Gram staining. Various biochemical tests; oxidase, indole production, methyl red, Voges Proskeur and citrate utilization were performed for biochemical identification of presumptive *P. aeruginosa* isolates.

Antibiotic susceptibility profiling

Antibiotic sensitivity of *P. aeruginosa* was evaluated by Kirby Bauer disk diffusion method using various antibiotics discs (OXOID) i.e. amikacin (30µg), colistin (10µg), ampicillin (10µg), meropenem (10µg),ceftazidim (30µg), ciprofloxacin (5µg) and tobramycin (10µg). The standard suspension of each strain was spread on Mueller Hinton agar plates. Antibiotic discs were dispensed on Muller Hinton agar medium containing *P. aeruginosa* isolates followed by incubation at 37°C. After 24 hours, diameter of inhibition zone around each antibiotic disc was measured and compared with CLSI standards to designate *P. Aeruginosa* isolates as resistant, intermediate or susceptible (CLSI, 2012; Melaku *et al.,* 2012).

Phenol Chloroform Method was used to extract DNA from the MDR *P. aeruginosa* isolates (Derakhshan *et al.,* 2013). DNA concentration was estimated using

Thermo Scientific Nano Drop™ 1000 Spectrophotometer. Table 1 shows the sequence of specific primer used to amplify *MEXT* gene from MDR *P. aeruginosa* isolates. Amplified PCR products were electrophoresed on 1% agarose gel (Ghadiri *et al.,* 2012).

Results

Purification & biochemical characterization of P. aeruginosa isolates

Samples plated on cetrimide agar plates showed pigment production (yellow colored colonies) (Fig.1). Bio characterization showed that all the forty-four *Pseudomonas* isolates were positive for both oxidase and catalase test. Out of forty-four isolates, all were negative for indole, methyl red and Voges Proskeur test. There was a change in color of Simmon's citrate agar from green to blue after inoculation of microorganisms showing positive reaction for citrate utilization test.

Table 1. Primers used for detection of *MEXT* gene of MDR *P. aeruginosa*.

Target	Primer Sequence 5' to 3'	Base pair	Reference
mexT	F: CAGCACCGCGGTGTTCCGCATCG R: ACGGTCTTGCGCTTGGCGTTGGC	216 bp	Dumas *et al* (2006)

Antibiotic sensitivity profiling

The antibiotic sensitivity profile revealed that out of 44 isolates, 12 were found to be resistant to ceftazidim, 10 isolates showed intermediate resistance and 22 isolates were sensitive to ceftazidim. In case of meropenem, 26 isolates were sensitive, 11 were resistant and 9 isolates showed intermediate resistance. The resistance of amikacin was well reported as out of 44 isolates, 27 were found resistant to amikacin, 6 showed intermediate resistance and 11 isolates were sensitive. Twenty-five isolates showed sensitivity to colistin, 15 were resistant and 4 showed intermediate resistance. For tobramycin 16 isolates showed sensitivity, 18 were resistant, while intermediate resistance was exhibited by 10 isolates. In case of carbenicillin, 17 isolates were resistant, 17 were sensitive and 9 were found to be intermediately resistant. Ciprofloxacin was found to be resisted by 24 isolates. 16 showed sensitivity and 3 were intermediately resistant (Fig.2).

Table 2. Percentage antibiotic sensitivity/resistance of various antibiotics to *P. aeruginosa* isolates collected from burned patients.

Antibiotics	Disc Content	Standard zone		Resistant (%)	Sensitive (%)
		≥ S (mm)	≤ R (mm)		
Ceftazidim	30 µg	18	14	34.1	65.9
Meropenem	10µg	19	15	25	75
Amikacin	30µg	17	14	61.4	38.6
Colistin	10µg	11	10	31.9	68.1
Tobramycin	10µg	15	12	40.9	59.1
Carbenicillin	100µg	21	14	40.9	59.1
Ciprofloxacin	5µg	21	15	56.8	43.2

The result of antibiotic sensitivity of all the 44 isolates to various antibiotics is described in Table 2 and Fig.3.

Molecular characterization

Among all the 44 *P. aeruginosa* isolates, 13 isolates were found to be multi-drug resistant. Two of these MDR isolates were selected for detection of *MEXT* gene by using specific primer through PCR. Amplified PCR product was electrophoresed on agarose gel. Fig.4 showed a band of 216bp size after electrophoresis.

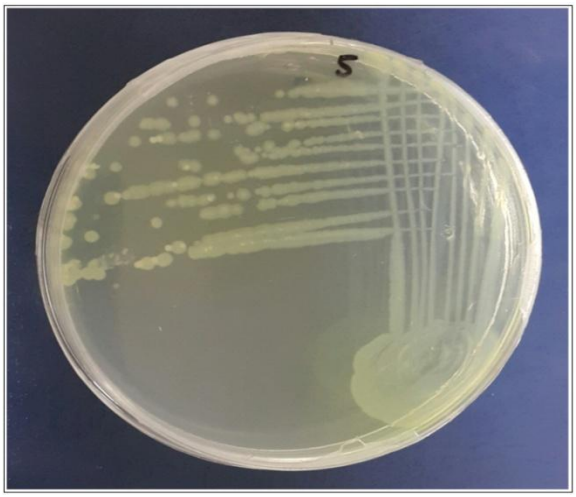

Fig. 1. *P. aeruginosa* growth on cetrimide agar plates showing yellowish pigment production.

Discussion

Isolation and purification of Pseudomonas aeruginosa

In the present study, total of 100 samples were collected from the burn ward of Allied hospital Faisalabad. The samples were collected from different burnt sites of the burnt patients and after processing in laboratory it was known that among the 100 samples, 44 isolates (44%) were positive for *P. aeruginosa*.

Fig. 2. Shows the antibiotic susceptibility pattern of *P. aeruginosa* to various antibiotics. a) colistin, b) meropenem, c) amikacin, d) ceftazidim. *P. aeruginosa* was most sensitive to meropenem, while least sensitive to amikacin.

This result was close to a study conducted in China where more than 50% prevalence of *P. aeruginosa* was observed during 2009-2011 (Hancock and Speert, 2000). During 2010, tertiary care hospitals of Karachi and Lahore had showed prevalence rate of 40% and 35.5%, respectively (Hirsch and Tam, 2010). In Saudi Arabia, a survey showed similar prevalence of 30.6% (Landman *et al.,* 2007). The present study showed the prevalence of 44% for *P. aeruginosa*. This might be due to poor hospital hygiene conditions and abuse of antibiotics without any clinical laboratory reports of culture sensitivities.

Antibiotic susceptibility profiling of P. aeruginosa:

In the present study, the results of antibiotic resistance for *P. aeruginosa* were as follows: colistin 31.82%, meropenem 25%, amikacin 61.36%, ceftazidim 34.09%, tobramycin 40.91%, carbenicillin 40.91%, and ciprofloxacin 56.82%. These results were closely related to a recent study done in India (Doring *et al.,* 2000), showing antibiotic resistance ratio for tobramycin 42.6%, 20% for ceftazidim, 40% for carbenicillin, 60% for amikacin while for colistin and meropenem, resistance was found to be 39.2% 27%, respectively (Pessoa-Silva *et al.,* 2003).

Another study conducted in Pakistan showed highest antibiotic resistance of *P. aeruginosa* (84.1%), followed by *E. coli* (68.5%) (Hope *et al.,* 2006). In another study, resistance for Colistin was reported to be 25.8% (Lindsay and VonHoly, 2006). Fadeyibi *et al* (2013), reported the similar results about antibiotics sensitivity as; tobramycin (60.4%), amikacin (73%), and ciprofloxacin (60%) (Fadeyibi *et al.,* 2013). Sensitivity to colistin in *P. aeruginosa* was also reported in Pakistan by Whiteley that can be used as alternative for meropenem in treatment of burn wound infection (Whiteley *et al.,* 2001).

It is reported that the infection caused by *P. aeruginosa* was more prevalent one due to high antibiotic resistance and this antibiotic resistance may be due to activation of different genes and operon systems that enable this bacterium to resist these antibiotics (Khan *et al.,* 2010). It is suggested to use both colistin and meropenem for treatment of burn wound infections due to *P. aeruginosa* (Pruitt *et al.,* 1983).

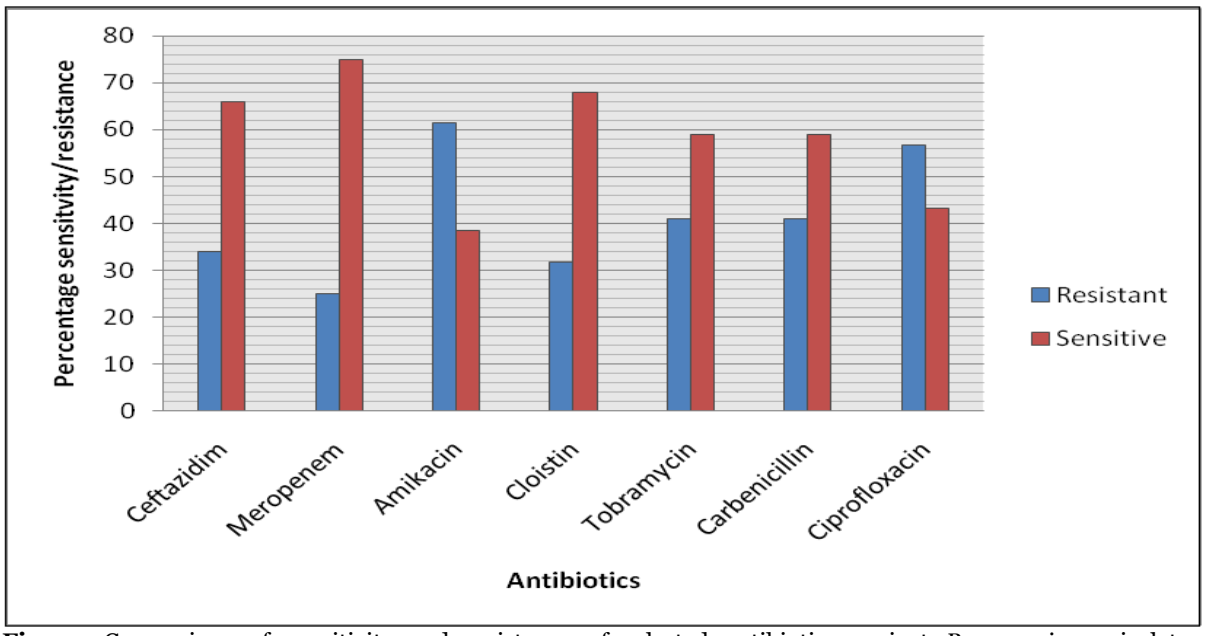

Fig. 3. Comparison of sensitivity and resistance of selected antibiotics against *P. aeruginosa* isolates. Meropenem showed highest sensitivity while amikacin least.

Molecular detection of MEXT gene of P. aeruginosa:
In the present study, out of 44 isolates, 13 isolates showed resistance to more than three antibiotics used being called as multidrug resistant (MDR) isolates. Among these MDR isolates, four were completely resistant to amikacin, tobramycin, carbenicillin, ciprofloxacin. While two showed resistance to amikacin, tobramycin and carbenicillin.

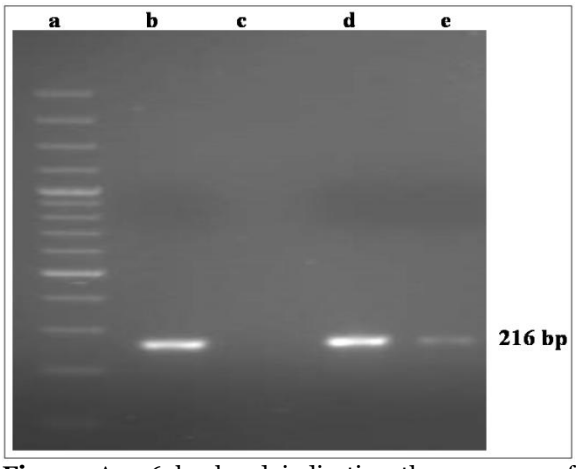

Fig. 4. A 216 bp band indicating the presence of *MEXT* gene in MDR *P. aeruginosa* isolates. Lane 'a' denotes DNA ladder (L), while b and c represent positive and negative control, respectively. Lane d and e were test samples (MDR *P. aeruginosa*) positive for 216 bp *MEXT* gene.

Three were resistant tobramycin, ciprofloxacin and colistin, while three isolates were resistant to all the antibiotics used in this study.

These results were closely resembled to a study by Hope *et al* (2006), who reported the resistance of *P. aeruginosa* to commonly used antibiotics including tobramycin, ciprofloxacin, meropenem and carbenicillin (Hope *et al.,* 2006; Pruitt *et al.,* 1983; Percival *et al.,* 2005). These MDR strains develop resistance due the activation of many operons and efflux pumps.

These pumps are activated by a number of genes. One of these genes is *MEXT* gene that activates MexEF-OprN which further activates an efflux pump that enables this MDR to develop antibiotic resistance. In the present study, out of 13 MDR isolates, 2 isolates (used for amplification of *MEXT* gene by PCR) showed a band of 216 bp. These results were in harmony with Pirnay *et al.* who identified this gene on agarose gel with 216bp length (Pirnay *et al.,* 2003). A major cause of resistance mechanism in *P. aeruginosa* may be due to the presence of MDR efflux system that enables this organism to resist a broad range of antibiotics. This MDR *P. aeruginosa* resist

almost all the newly synthesized antibiotics (Livermore, 2002).

Conclusion

In conclusion, multidrug resistance *P. aeruginosa* is a major issue in the nosocomial infections and colistin and meropenem may be effective in treating *P. aeruginosa* infections. Activation of *MEXT* gene in MDR *P. aeruginosa* may be one of the factors leading to antibiotic resistance to a broad range of antibiotics.

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References

CLSI C. 2012. M100-S25: Performance Standards for Antimicrobial Susceptibility Testing. Twenty-Fifth Informational Supplement.

Derakhshan S, Peerayeh SN, Fallah F, Bakhshi B, Rahbar M. 2013. Identification of Extended Spectrum Beta-lactamase producing Klebsiella pneumoniae isolated from Intensive Care Unit (ICU) patients in three hospitals in Tehran. Infection, Epidemiology and Medicine **1,** 9–13.

Doring G, Conway SP, Heijerman HG, Hodson ME, Hoiby N, Smyth A, Touw DJ. 2000. Antibiotic therapy against Pseudomonas aeruginosa in cystic fibrosis: a European consensus. European Respiratory Journal **16,** 749-67.

Drenkard E. 2003. Antimicrobial resistance of Pseudomonas aeruginosa biofilms. Microbes and Infection **5,** 1213-1219.
https://doi.org/10.1016/j.micinf.2003.08.009

Fadeyibi IO, Raji MA, Ibrahim NA, Ugburo AO, Ademiluyi S. 2013. Bacteriology of infected burn wards of a teaching hospital in Southwest Nigeria **39,** 168-173.
https://doi.org/10.1016/j.burns.2012.02.005

Ghadiri H, Vaez H, Khosravi S, Soleymani E. 2012. The antibiotic resistance profiles of Bacterial strains isolated from patients with hospital-acquiredbloodstream and urinary tract infections.Critical CareResearch and Practice **2,** 797-890.
http://dx.doi.org/10.1155/2012/89.0797.

Hancock RE, Speert DP. 2000. Antibiotic resistance in Pseudomonas aeruginosa: mechanisms and impact on treatment. Drug Resistance Updates **3,** 247-255.
https://doi.org/10.1054/drup.2000.0152

Hirsch EB, Tam VH. 2010. Detection and treatment options for Klebsiella pneumoniae carbapenemases (kpcs): an emerging cause of multidrug-resistant infection. Jornal of Antimicrobial Chemotherapy **65,** 1119–25.
https://doi.org/10.1093/jac/dkq108

Hope R, Warner M, Potz NC, Fagan EJ, James D, Livermore DM. 2006. Activity of tigecycline against ESBL-producing and AmpC-hyperproducing Enterobacteriaceae from South-East England. Journal of Antimicrobial Chemotherapy **58,** 1312–4.
https://doi.org/10.1093/jac/dkl414

Khan E, Ejaz M, Zafar A, Jabeen K, Shakoor S, Inayat R. 2010. Increased isolation of ESBL producing Klebsiella pneumoniae with emergence of carbapenem resistant isolates in Pakistan: Report from a tertiary care hospital. Journal of Pakistan MedicalAssociation **60**,186–90.

Landman D, Bratu S, Kochar S, Panwar M, Trehan M, Doymaz M, Quale J. 2007. Evolution of antimicrobial resistance among Pseudomonas aeruginosa, Acinetobacter baumannii and Klebsiella pneumoniae in Brooklyn, NY. Journal of Antimicrobial Chemotherapy **60**, 78–82.

Lindsay D, VonHoly A. 2006.Bacterial biofilms within the clinical setting: What healthcare professionals should know. Journal of Hospital Infection **6,** 1-28.

Livermore DM. 2002. Multiple Mechanisms of Antimicrobial Resistance in Pseudomonas aeruginosa: Our Worst Nightmare?. Clinical Infectious Diseases **34,** 634-640.

Maseda H, Saito K, Nakajima A, Nakae T. 2000.Variation of the mexT gene, a regulator of the MexEF-OprN efflux pump expression in wild-type strains of Pseudomonas aeruginosa. FEMS Microbiology Letters **192,** 107–12. https://doi.org/10.1111/j.1574-6968.2000.tb09367.x.

Mayhall CG. 2003. The Epidemiology of Burn Wound Infections: Then and Now. Clinical Infectious Diseases **37,** 543-550. https://doi.org/10.1086/376993

Melaku S, Kibret M, Abera B. 2012. Antibiogram of nosocomial urinary tract infections in Felege Hiwot referral hospital.Ethiopia **12.** http://dx.doi.org/10.4314/ahs.v12i29

Percival SL, Bowler PG, Russell D. 2005. Bacterial resistance to silver in wound care. Journal of Hospital Infection **60**, 1-7.

Pessoa-Silva CL, Moreira BM, Almeida VC, Flannery B, Lins MCA, Sampaio JLM. 2003. Extended-spectrum beta-lactamase-producing Klebsiella pneumoniae in a neonatal intensive care unit: risk factors for infection and colonization. Journal of Hospital Infection **53,** 198–206.

Pirnay JP, Bilocq F, Pot B, Cornelis P, Zizi M, Van Eldere J, Deschaght P, Vaneechoutte M, Jennes S, Pitt T, De Vos D. 2009. Pseudomonas aeruginosa population structure revisited. PloS one **4,** e7740.

Pruitt BA, Lindberg RB, McManus WF, Mason AD. 1983. Current approach to prevention and treatment of Pseudomonas aeruginosa infections in burned patients. Infectious Diseases **5,** 889-897.

Richard P, Floch RL, Chamoux C, Pannier M, Espaze E, Richert H. 1994. Pseudomonas aeuroginosa Outbreak in a Burn Unit: Role of Antimicrobials in Emergence of Multiply Resistant Strains. Infectious Diseases **170,** 377-383.

Schweizer HP. 2003. Efflux as a mechanism of resistance to antimicrobials in Pseudomonas aeruginosa and related bacteria: unanswered questions. Genetics and Molecular Research **2,** 48–62. https://doi.org/10.3923/pjbs.2007.924.927.

Stover CK, Pham XM, Erwin AL, Mizoguchi SD, Warrener P, Hickey MJ, Brinkman FS, Lory S, Olson MV. 2000. Complete genome sequence of Pseudomonas aeruginosa PA01,an opportunistic pathogen. Nature **406,** 959-964. https://doi.org/10.1038/35023079.

Waszczuk K, Gula G, Swiatkowski M, Olszewski J, Herwich W, Drulis-Kawa Z, Gutowicz J, Gotszalk T. 2012. Evaluation of Pseudomonas aeruginosa biofilm formation using piezoelectric tuning fork mass sensors. Sensors and Actuators B: Chemical **170,** 7-12.

Whiteley M, Bangera MG, Bumgarner RE, Parsek MR, Teitzel GM, Lory S, Greenberg EP. 2001. Gene expression in Pseudomonas aeruginosa biofilms. Nature **413,** 860-864.