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Frequency of mexa gene in pseudomonas aeruginosa isolated from clinical samples of a Tertiary Care Hospital in Pakistan.

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ABSTRACT… Objectives: This study was designed to detect the MexA gene of Pseudomonas aeruginosa resistant strains in tertiary care hospital, Peshawar. **Study Design:** Cross-sectional study. **Setting:** Department of Pathology, Khyber Teaching Hospital, Peshawar, Pakistan. **Period:** 14 months duration from April 2015 to May 2016. **Material & Methods:** The specimens including burn wound swabs, pus and urine were obtained from different patients and were processed on blood agar and MacConkey medium for isolation and identification. Conventional PCR was performed for the MexA gene on 50 specimens. **Results:** The simple conventional PCR was done for MexA (The Mex AB OprM operon resistance genes) and the O-antigen acetylase gene (the species-specific gene) separately, gave positive bands for 49 out of the 50 specimens. Our finding confirms the presence of the MexA gene (and hence most probably MexABOprM operon) in 49 out of 50 specimens of Pseudomonas aeruginosa. **Conclusion:** Among other resistance mechanisms to antibiotics and disinfectants, the MexABOprM efflux pump might have a role.

Key words: Frequency, Mex A, Peshawar, Pseudomonas.

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INTRODUCTION

Pseudomonas aeruginosa is a gram-negative aerobic coccobacillus. The usual sites of Pseudomonas aeruginosa infection are tissues with low immunity, which further leads to inflammation and sepsis. The infection, if severe or a vital part is involved may lead to high mortality. The organism early colonize the wet surface of medical equipment including catheters. It often causes infections in hospitals.[1,2]

The most common species of Pseudomonas infecting humans is Pseudomonas aeruginosa. The infections may be of blood, urinary tract, respiratory tract (pneumonia) and surgical infection; which may be fatal.[3]

Among the microorganisms Pseudomonas aeruginosa genome contains the largest number of resistance genes i-e 50 antibiotic resistance genes. So, it has an immense potential to develop antibiotic resistance.[4,5]

There is reported resistance to all antibiotics off and on from over the world. The ratio of resistance to different antibiotics may depend upon the extensive use of an antibiotic (amikacin, gentamicin, and ciprofloxacin) and the costly and non-available antibiotics of choice.[6,7]

Among the drug resistance systems; the important is, enhancement in the expression of enzymes that degrade drugs, decreased production of outer membrane proteins, accelerated expression of efflux pumps, drug modifying, and target sites mutation/change.[8] The drug resistance in Pseudomonas aeruginosa is also linked to change in gene expression such as Mex drug efflux pumps.[9] It is observed that there is a 4-8 fold increase in the expression of MexA, MexB and OprM genes in Pseudomonas aeruginosa when grown in sub-inhibitory concentrations of antibiotics. There are four types of drug efflux pump systems. The type of the pump system is based on the membrane through which the

protein is transported.[10,11]

In P. aeruginosa four medically important multi-drug efflux pump systems are described. These Mex pumps all belong to Resistance nodulation cell division (RND) family and are alike in genetic makeup but they are different in substrate and regulation. There is a lot of genetic polymorphism in P. aeruginosa.[12] This is due to mutations and horizontal gene transfer. Due to the genetic polymorphism, Pseudomonas aeruginosa shows phenotypic differences in metabolism, virulence factors, and regulation of different genes.[13] Resistance to antibiotics is the combined result of multi-drug efflux pumps and other mechanisms. The acquired resistance can be due to mutation in the genes located in chromosomes.[14] Polymyxins, carbapenems and tigecycline considered as active drugs for Pseudomonas aeruginosa have also developed resistance.[15] It provided comprehensive data for the prevention of this infection and to target a new gene.

This current study also was designed to determine the frequency of the MexA gene in Pseudomonas aeruginosa resistant strains in the tertiary care hospital of Peshawar.

MATERIAL & METHODS

Between April 2015 and May 2016, 50 non-replicative, consecutive samples were collected from clinical isolates of Khyber Teaching Hospital, a tertiary care hospital in Peshawar. A majority of the isolates were obtained from a wound and burn/ skin infections. The specimens were inoculated on, a blood agar plate and selective differential medium MacConkey medium. Identification was carried out by Gram stain, urease, citrate, oxidase test, and Triple Sugar Iron (TSI) assay.

Drug susceptibility of the bacterial isolates was tested on Mueller-Hinton agar (MHA) (CM337-Oxoid, England). MHA medium was inoculated with the test organism, and filter paper discs (Oxoid., Eng) with the antibiotics to be tested were placed on the medium. The sensitive antibiotic produces clear zone, due to inhibition of the organism according to clinical laboratory standard protocol (CLSI).[16]

DNA was extracted from the clinical isolates according to the kit manufacturer protocol using Thermo Scientific "Gene JET Genomic DNA Purification Kit" (Thermo Scientific, Waltham, MA).[17]

Conventional PCR was performed on the 50 clinical isolates of Pseudomonas aeruginosa for two genes separately using specific primers; according to the kit manufacturer protocol. Two primers were used; one was species-specific O-antigen acetylase gene and for the MexA gene. Details of primers are listed in Table-II.

All the isolates were screened for the selective species-specific O-antigen acetylase gene and MexA genes in pathogenic P. aeruginosa. The list of primers and optimized PCR conditions are shown in Table-I. The amplified products were electrophoresed at 110 volts for 40 to 60 minutes on 1.5% agarose suspended in 1X TAE buffer. Gels were stained with Ethidium Bromide solution. Bands were visualized and photographed by the gel documentation system. The amplicon sizes were determined by comparing them with a 50-bp DNA ladder (Thermo scientific).[17]

RESULTS

The drug sensitivity pattern of the samples is as follows,

(%) percentage

(P. Sp) Resistant Species

(Σ. Sp) Sensitive Species

The simple conventional PCR was done for MexA (The Mex AB OprM operon resistance gene) and O antigen acetylase gene (the species-specific gene) separately. Mex A was detected in 49 (98%) out of 50 isolates (L13 missed for Mex A while antigen acetylase was identified in 48 (96%) out of 50 specimens (L14 and L9 missed for OAA) shown in Figure 1.

DISCUSSION

The study is well in co-relation with the result of the study conducted by Dumas et al., 2005 from the Swiss National Science Foundation, in 2005; in which the expression of MexABOprM operon was enhanced in resistant laboratory and

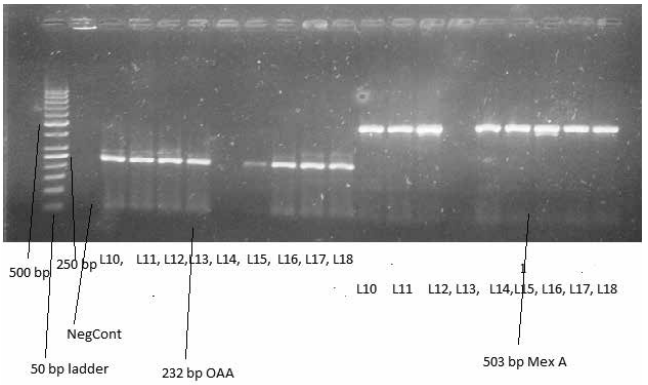

Figure-1. Gel electrophoresis of PCR products of MexA (503bp) and O antigen acetylase (232bp).

clinical strains.[18] A four to eightfold increase in the expression of mexA, mexB, and oprM genes was observed in nalB mutants, in which there is overexpress of OprM. MexX and mexY genes were induced eight to 12 times in the presence of 2 mg/ L tetracycline. The mexC/oprJ and mexE/ oprN gene expression levels were increased 30 to 250 fold and 100 to 760 fold in nfxB and nfxC mutants, respectively; in antibiotics exposed strains.[19,20]

Drug Disc	Abbreviation	Strength	Resistance zone
Ciprofloxacin	CIP	05ug	≤15 mm
Imipenem	IPM	10ug	≤13 mm
Meropenem	MEM	10ug	≤13 mm

Table-I. Antibiotics disc and standard strength and resistance zone.

Gene	Primer sequence 5' -3'	Base pairs	Size bp	Annealing T °C
O-antigen acetylase gene	F: CTGGGTCGAAAGGTGGTTGTTATC R: GCGGCTGGTGCGGCTGAGTC	24 20	232	63
MexA(3)	F: CTCGACCCGATCTACGTC R: GTCTTCACCTCGACACCC	18 18	503	57

Table-II. Oligonucleotide sequence of primers used for the molecular characterization of antibiotic-resistant genes of Pseudomonas aeruginosa.

Antibiotic	Total Specimens	% age of R. Sp	% age of Sen. Sp	R.Sp in 50	S. Sp in 50
Ciprofloxacin	50	54	46	27	23
Meropenem	50s	13	87	6	44
Ceftazidime	50	53	47	26	24

Table-III.

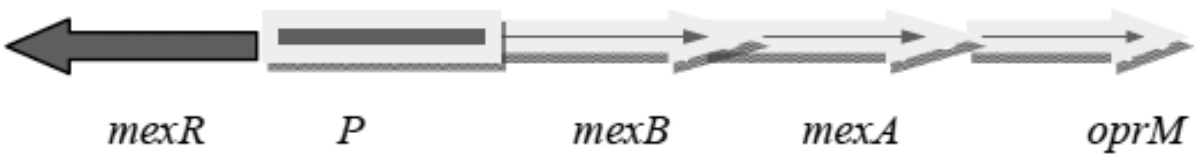

Figure-2. MexAB-open efflux system operon organization in P. aeruginosa.

The unnecessary use of antibiotics may cause many folds increase in the expression of MexABOprM operon. It indicates that the organism operates/regulates the efflux pump capacity per its environmental needs and blocking/down-regulation of the pump that might be used to get rid of at least one resistance mechanism of this problematic organism.[21]

In a study, the occurrence of MexAB-OprM efflux Pump Operon on Septicaemic Pseudomonas aeruginosa chromosome by Grawi et al., 2012 in Iraq (2012) reported 53 specimens of Pseudomonas aeruginosa isolated from

patients of septicemia. In the above study, all the specimens of Pseudomonas aeruginosa were found positive for Mex A and B and Mex R genes (the regulatory gene of MexABOprM operon).[21]

The current study is also supported by the findings from the USA, in which they pointed the inherited resistance of Pseudomonas aeruginosa to many frontline antibiotics is due to other mechanisms like low outer membrane permeability of drug pumps of RND family. In RND pump family Mex AB OprM is of major importance.[22] There is every chance that any mechanism may be lost due to mutation. So, mutation may occur in the Mex AB OprM operon in a given population.[23]

CONCLUSION

The MexA gene is a constitutive chromosomal gene. The detection/degree of expression will provide a guide to the use of multidrug efflux pump inhibitors for the treatment of Pseudomonas aeruginosa. An example of efflux pump inhibitors for P. aeruginosa is are peptidomimetics. The substrates include all antibiotic classes and antibacterial specificity is very wide. Commercial Kits are already available in the market to detect the degree of expression of MexABOprM operons. This will help clinicians to treat the infections properly.

Conflict of Interest

The authors declare no conflict of interest.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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2	Amina Gul	Data analysis and interpretation of data.	
3	Jawad Ahmed	Study design and sample collection.	
4	Noor Rehman	Sample processing and Data analysis.	
5	Liaqt Ali	Study writeup and Critical review.	
6	Shahina Mumtaz	Study design and critical review.	
7	Anees Muhammad	Data analysis, interpretation and Final Draft.	