Occurrence of MexAB-OprM Efflux Pump Operon on Septicemic Pseudomonas Aeruginosa Chromosome

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ABSTRACT:

BACKGROUND:

P. aeruginosa remains an important cause of life-threatening bloodstream infection in immunocompromised patients, particularly those with hematologic malignancies complicated by neutropenia. One of the most worrisome characteristics of P. aeruginosa consists is its low antibiotic susceptibility This low susceptibility is attributable to concerted action of chromosomally-encoded multidrug efflux pumps genes. These genes are often controlled by regulatory gene located on the same operon of efflux pump. One of particular significance is the MexAB - OprM efflux system, which is expressed constitutively, thereby contributing to the well-known intrinsic resistance of this organism to multiple antimicrobials.

OBJECTIVE:

To detect the occurrence of mexAB-OprM operom on the chromosomes of septicemic *P. aeruginosa*(SPA).

METHODS:

This study was include 53 *Pseudomonas aeruginosa* isolates isolated from patients their ages ranging from two days to 73 years,28 males and 25 females. Some of the isolates were isolated from acute, 15(28.3%), and chronic, 7 (13.2%), leukemic patients, 5 (9.4%) from each lymphoma and gastrointestinal neoplasms patients. Nine (17%), 3(5.7%), 6 (11.3%) and 3(5.7%) from urogenital neoplasms, breast cancer patients, septicemic patients due to burn infections and neonatal septicemia respectively. Chromosomal DNA was extracted from SPA isolates and subjected to PCR to amplify three genes of mexAB-OprM efflux pump.

RESULTS:

Multiplex PCR of mexAB-OprM efflux pump genes revealed that 53 (100%) were positive to all three genes of operon, *mexA, mexB* and the regulatory gene, *mexR.*

CONCLUSION:

P. aeruginosa can cause septicemia in cancer patients and other compromised patients, like patients suffering from extensive burns and neonatal infants. mexAB-OprM efflux pump genes are a chromosomal encoded genes and can be used as a markers in identification of SPA by molecular methods. These genes can be used individually or collectively in rapid identification of SPA, and rapid detection for mexAB-OprM efflux pump occurrence on their chromosomes.

KEYWORDS: mexAB-oprm efflux pump. septicemic pseudomonas. aeruginosa.

INTRODUCTION:

Microorganism present in circulating blood are a threat to every organ in the body . Microbial invasion of the bloodstream can have serious immediate consequences , including shock, multiple organ failure, disseminated intravascular coagulation, and death ,with mortality rates ranging from 30% to 50% . Positive blood cultures may help provide a clinical diagnosis as well as a specific etiological diagnosis [1,2].

P. aeruginosa remains an important cause of life threatening bloodstream infection in immunocompromised patients, particularly those with hematologic malignancies complicated by neutropenia [3]. septicemia is frequently iatrogenic and is usually seen in hospitalized patients with various comorbid conditions.

Bloodstream infection may be primary or secondary to a discrete focus of infection. Common primary sites of infection include the

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urinary and gastrointestinal tracts, lungs, skin and soft tissues, and intravascular foci, including indwelling central venous catheters[4].

Blood stream invasion and dissemination of *Pseudomonas* from local sites of infection is probably mediated by the same cell associated and extracellular products responsible for the localized disease [5].

One of the most worrisome characteristics of *P. aeruginosa* consists is its low antibiotic susceptibility. This low susceptibility is attributable to a concerted action of multidrug efflux pumps withchromosomally encoded antibiotic resistance genes and the lowpermeability of the bacterial cellular envelopes. In addition to thisintrinsic resistance, *P. aeruginosa* easily develops acquired resistance to structurally and functionally dissimilar antibiotics by mutation in chromosomally-encoded genes of antibiotic resistance determinants. Development of multidrug resistance by *P. aeruginosa* isolates requires several different genetic events that include acquisition of different mutations and/or horizontal transfer of antibiotic resistance genes [6]

Bacterial multidrug efflux pumps play an important role in the antimicrobial resistance of gram – negative pathogens, particularly *Pseudomonas aeruginosa*, where six multidrug efflux systems (MexAB-OprM, MexCD-OprJ, MexEF-OprN, MexXY-OprM, MexJK-OprM, and MexVW-OprM) are discovered. Of particular significance is the MexAB - OprM efflux system, which is expressed constitutively, thereby contributing to the well-known intrinsic resistance of this organism to multiple antimicrobials [7,8]. MexAB – OprM is central to both intrinsic and mutational multidrug resistance [9].

PATIENTS ,MATERIALS AND METHODS:

This study was include 53 *Pseudomonas aeruginosa* isolates isolated from patients their ages ranging from two days to 73 years,28 males and 25 females. Some of the isolates were isolated from acute, 15(28.3%), and chronic, 7 (13.2%), leukemic patients, 5 (9.4%) from each lymphoma and gastrointestinal neoplasms (gastric cancer and colon cancer) patients. Nine (17%), 3(5.7%), 6 (11.3%) and 3(5.7%) from urogenital neoplasms, breast cancer patients, septicemic patients due to burn infections and neonatal septicemia respectively(table 1).

All of these isolates were collected in a period from February to December 2010, and were reidentified using biochemical tests and according to Forbes *et al*, 2002[10].

Chromosomal DNA was extracted from all isolates using commercially available DNA extraction kit (Promega-USA). Chromosomal, and PCR products DNA were resolved by horizontal agarose gel electrophoresis. Agarose at concentrations of 1.5%, and 0.8% was prepared for multiplex PCR products and chromosomal DNA electrophoresis respectively. Purity and concentration of and chromosomal DNA extracts were measured via ultraviolet spectrophotometric determination method [11].

The occurrence of three genes of efflux pump, *mexA*, *mexR*, and *mexB*, were detected via multiplex PCR procedures using oligonucleotide primers[12]. Table (2) shows the sequence and molecular weight of PCR products of three genes.

Table 1: Causes of Pseudomonas aerugonosa septicemia

Cause of septicemia	No.of isolates	Percentage of isolates(%)
Acute leukemia	15	28.3
Chronic leukemia	7	13.2
Lymphoma	5	9.4
Urogenital neoplasms	9	17.0
Gastrointestinal neoplasms	5	9.4
Breast cancer	3	5.7
Burn infections	6	11.3
Neonatal septicemia	3	5.7
Total	53	100

Table 2: Sequence and molecular weight of PCR products of *mexA*, *mexR* and *mexB* genes of SPA.

gene	Sequence of forward Primer(5´- 3´)	Sequence of reverse primer (5´- 3´)	Product bp
mexA	CTCGACCC GATCTACGTC	GTCTTCACCTCGACACCC	503
mexR	GAACTACCCCGTGAA TCC	CACTGGTCGAGGAGATGC	411
mexB	TGTCGAAGTT TTTCATTGATAG	AAGGTCAC GGTGATGGT	280

Go-Taq green master mix	12.5 µl
Each primer (three sets of 10µM each)	1.5 µl
Nuclease free distilled water	2.5 µl
DNA template(20µg)	1µl

Multiplex PCR was performed using Go-Tag green master mix kit (promega-USA) as follows: in an Eppendorf reaction tube, 25 µl master mix wasprepared for each test .A master mix contained the following components(according to them anufacturerinstruction)The cycling was performed using protocol comprising an initial denaturing step at 94°C for 3 minutes, followed by 32 cycles of 94°C for 30 seconds,57°C for 45 seconds and 72°C for 1 minute [12].

RESULT:

Chromosomal DNA was extracted and then subjected to agarose gel electrophoresis. Figure (1) shows agarose gel electrophoreticogram of chromosomal DNA extracted from SPA isolates. The extracted DNA was then subjected to multiplex PCR, and the product was agarose electrophoresed. Figure (2) shows agarose gel electrophoreticogram of *mexA. mexR* and *mexB* PCR products. All of lanes show three bands of *mexA* with molecular weight of 503bp, *mexR* PCR products were 411bp and *mexB* were 280bp.

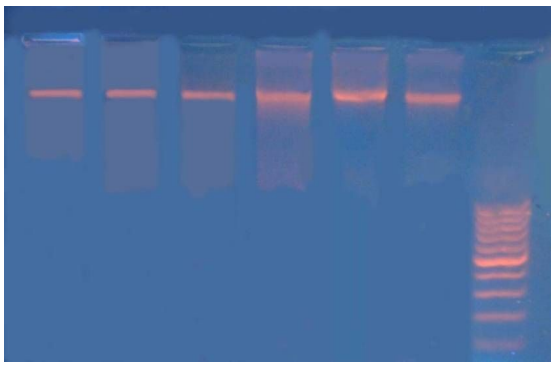

Figure 1: Agarose gel electrophoreticogram of chromosomal DNA of septicemic Pseudomonas aeruginosa.

On the right 1kb molecular ladder, other bands represent the chromosomal DNA of septicemic *Pseudomonas aeruginosa* isolates.

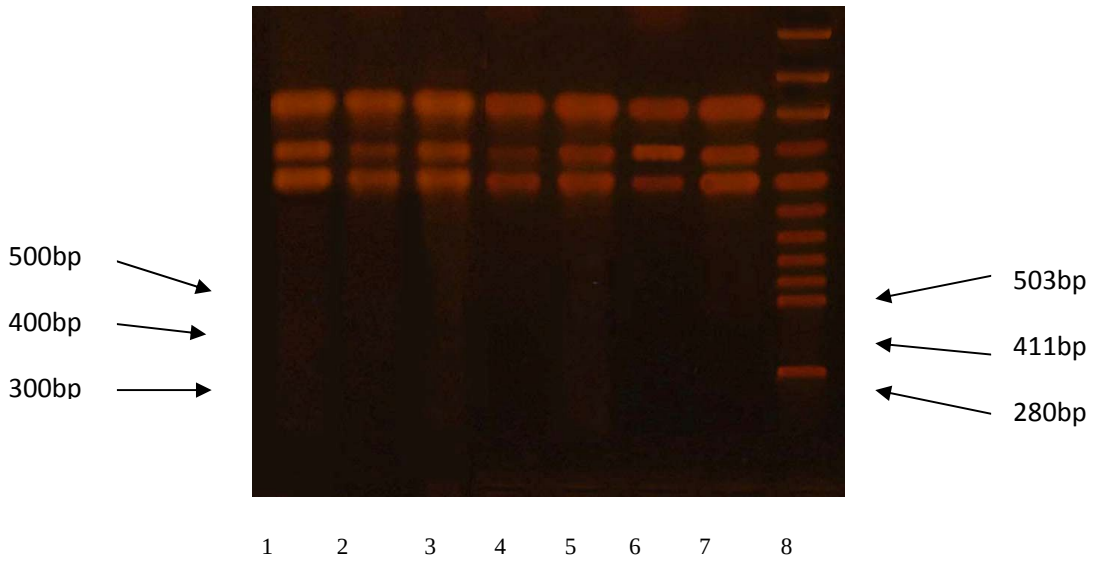

Figure 2: Agarose gel electrophoreticogram of *mexA*, *mexR* and *mexB* PCR products.

Lane 1 is 100bp molecular ladder, 2,3,4,5,6,7,8 lanes are PCR products. All show three bands of, *mexA*(503bp), *mexR*(411bp)and *mexB*(280bp).

All of the SPA (53,100%) have three genes of, *mexA*, *mexR* and *mexB* genes on their chromosomes.

Table 3: Numbers and percentages of occurrence of mexA, mexR and mexB genes on chromosomes of septicemic Pseudomonas aeruginosa isolates.

Gene	No. of positive isolates	% of positive isolates	No. of negative isolates	% of negative isolates
mexA	53	100	0	0
mexR	53	100	0	0
mexB	53	100	0	0

DISCUSSION:

The MexA, MexB, and OprM of mexAB-OprM efflux pump of *P. aeruginosa* are subunits were assumed to function as the membrane fusion protein, the body of the transporter, and the outer membrane channel protein, respectively. The global MexA structure showed unforeseen new features with a spiral assembly of six and seven protomers that were joined together at one end by a pseudo 2-fold image. The MexA subunit connected MexB and OprM, indicating that MexA is the membrane bridge protein[7].

The MexB subunit is central to the pump function, which spans the cytoplasmic membrane 12 times, selects antibiotics to be exported, and is assumed to transport the substrates expending the energy of the proton gradient across the cytoplasmic membrane[9,13]. The OprM subunit is the outer membrane - anchored lipoprotein that is assumed to play a role in the final step of antibiotic extrusion facilitating the exit of antibiotic across the outer membrane the entire protein moiety of MexA protruded to the periplasmic aqueous space[9]. Figure (3) shows the assembly of mexAB-OprM efflux pump of *P. aeruginosa.*

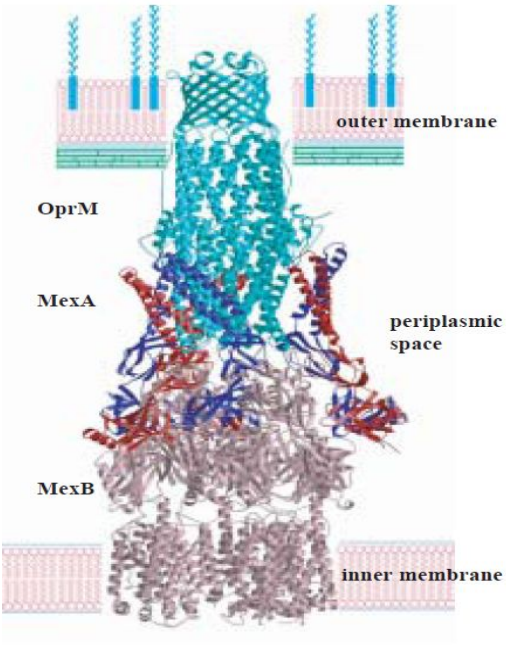

Figure 3: Proposed assembly model of MexAB-OprM antibiotic efflux pump [9].

The genes that encode for these proteins are chromosomal, *mexA, mexB* and *oprM* genes, and their operon contain another gene, regulatory gene, *mexR* gene. in the opposite orientation capable of encoding a polypeptide with apredicted molecular mass of 16,964 Da. Disruption of this gene, *mexR*, is associated with upregulation of the *mexAB-oprM* operon in both clinical and laboratory strains[14]. Figure (4) shows genetic organization of the *mexAB-oprM* efflux system operon in *P. aeruginosa*.

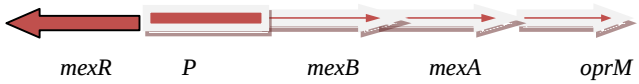

Figure 4: Genetic organization of the mexAB-oprM efflux system operon in P. aeruginosa.

MexR binding to the *mexR-mexA* intergenic region at promoter site (P) directly represses *mexAB-oprM* expression and autoregulates *mexR*. P is a protein modulator of MexR, which directly binds it, inhibiting repression of *mexAB-oprM*.[15].

Basically, an important situation can lead to opportunistic infections caused by *P. aeruginosa*. Immunosuppression of a patient can allow strains with low pathogenicity to invade tissues. In cancer patients, when counts of polymorphonuclear cells are below 100/mm[3] of peripheral blood, *P. aeruginosa* septicemia occurs more frequently [16]. Most of the patients of this study suffering from cancers, especially leukemia. In this study we investigate the occurrence of most of *mexAB-oprM* efflux system operon on the chromosomes of *P. aeruginosa*, that isolated from patients with compromised conditions. All of the *P. aeruginosa* isolates that obtained from them were positive to all three genes tested. The MexAB-oprM efflux system contributes to the natural resistance of bacteria to a wide range of antibiotics including fluoroquinolones, β -lactams, and β-lactamase inhibitors[17]. efflux systems in *P. aeruginosa* might be also critical for the efflux of virulence factors, in addition to their established role of exporting harmful substances such as antibiotics or detergents[18].Because of that, all of SPA have *mexAB-oprM* efflux system operon on their chromosomes, SPA can be rapidly and easily identified by amplification of one or more of this operon using multiplex or conventional PCR.

CONCLUSION:

We found that, *P.aeruginosa* can cause septicemia in cancer patients and other compromised patients, like patients suffering from extensive burns and neonatal infants. *mexAB-oprM* efflux system operon is a chromosomal encoded feature occurs in

all of SPA chromosomes and can be used as a marker in identification of SPA and give an idea about a possible emergence of antibiotic resistance. PCR can shorten the time of identification to few hours.

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