SHORT REPORT

Determination of Qnr allele frequencies in Fluoroquinolone resistant Pseudomonas aeruginosa isolated from burn wounds

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Abstract

Effective treatment of burn wound infections is precluded by high prevalence of nosocomial infections coupled with high resistance rates against commonly used antimicrobials. Pseudomonas aeruginosa is an important nosocomial pathogen in burn infections due to high morbidity and mortality associated with it. We analyzed fluoroquinolone resistant Pseudomonas aeruginosa for the presence of Qnr gene, owing to high genetic mobility of this plasmid-borne gene. Bacteria were isolated from burn wound patients from Pak-Italian ModernBurn Center, Nishter Hospital Multan. Fluoroquinolone resistant isolates were selected and PCR-based assay was designed to determine the frequencies of different alleles. QnrB was found in highest number of isolates (10.8%) followed by QnrD (6%). We could not find any QnrC positive sample. As far as we could search, this is the first report on detection of Qnr gene in Pseudomonas aeruginosa from Pakistan.

Keywords: Pseudomonas aeruginosa, Qnr Gene, Pentapeptide Repeat Protein, Fluoroquinolone, Antibiotic Resistance

Introduction

Around 0.3 million people die annually because of burn wounds around the globe with nosocomial infections contributing in 50% of these deaths.[1] Gram negative, non-spore forming organism, Pseudomonas aeruginosa is responsible for 10-20% hospital acquired infections and causes 40-75% mortality in burn patients.[2] Broad spectrum antimicrobial drugs, fluoroquinolones (FQn), are commonly suggested against pseudomonal infections.[3] Misuse of FQn has led to an increase in bacterial resistance due to chromosomal mutation in DNA gyrase (gyr A and gyr B genes) and topoisomerase IV or changes in expression of genes controlling transport of FQns.[4]

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Another important acquired mechanism of FQn resistance is Qnr genes. They code pentapeptide repeat proteins which inhibit the action of FQn against bacterial DNA gyrase and topoisomerase IV.[5] Qnr genes are classified as QnrA, QnrB, QnrC, QnrD and QnrS which differ 70% or more on the basis of nucleotide sequence.[6]

Owing to importance of Qnr based resistance in this pathogen, current study was designed to investigate presence of Qnr gene and determine relative frequencies of its alleles in FQn resistant isolates. Pseudomonas aeruginosa was isolated from burn wound infections. PCR based analysis of FQn resistant organisms was carried out to detect presence of Qnr alleles. Amongst all Qnr alleles, Qnr-B allele was found in highest number of isolates.

Methods

A total of 150 burn wound aspirates were collected by sterile syringes from 50 burn patients of both sexes (Male & Female) with an age range of 1.5 to 65 years during November 2016 to March 2017 at Pak Italian Modern Burn Center (PIBC), Nishtar hospital Multan. Aspirates were transported in sterile tubes containing Tryptic Soy Broth (TSB) within an hour to Microbiology lab of Institute of Pure and Applied Biology (IPAB) Bahauddin Zakariya University, Pakistan. They were cultured for 6h and streaked on Cetrimide Agar (CA) for isolation of Pseudomonas aeruginosa. A series of conventional biochemical tests such as oxidative fermentative, oxidase, nitrate reduction, arginine decarboxylase and indole test was performed for confirmation of isolated colonies of Pseudomonas aeruginosa.[7]

Identified colonies were streaked onto Muller Hinton Agar plates containing 8ug/mL of Levofloxacin to select FQn resistant isolates as per CLSI instructions.[8] Resistant colonies were directly transferred into PCR tubes.

PCR was performed for detection of qnr genes. The list of Primers are shown in the table. For each sample, 50µl PCR mix (25µl Taq polymerase, 5µl reverse & 5µl forward primers, 9µl de-ioni zed water, inoculum) was prepared.

Table-1: (Quinolone Resistant Primers (OLIGO / macrogen).

Primer	Forward Primer	Reverse Primer	Size (bp)
QnrA	ATTTCTCACGCCAGGATTTG	TGCCAGGCACAGATCTTGAC	574
QnrB	CTCTGGCRYTNGTYGGCGAA	TTYGCBGYYCGCCAGTCGAA	505
QnrC	GGGTTGTACATTTATTGAATCG	CACCTACCCATTTATTTTCA	307
QnrD	CGAGATCACTTTACGGGGAATA	AACAAGCTGAAGCGCCTG	583
QnrS	ACTGCAAGTTCATTGAACAG	GATCTAAACCGTCGAGTTCG	433

All this process was performed in ice box. For each primer (QnrA, QnrB, QnrC, QnrD, QnrS) amplification was executed as: single initial denaturation and bacterial lysis at 95°C for 10 minutes, followed by 35 cycles of denaturation at 95°C for 40 seconds, annealing temperature for QnrA, QnrB was 54°C, QnrC 50°C, QnrD 52°C, and QnrS 51°C, elongation at 72°C for 40 seconds followed by single final elongation at 72°C for 10 minutes. Amplified PCR products were analyzed on 1.5% agarose gel by using gel electrophoresis technique and examined under the UV transilluminator. SYBR-safe (Thermo-fisher, S33102) was used for visualization of DNA instead of ethidium bromide.

Resuls

Purpose of this study was to analyse the presence of Qnr genes in FQn resistant isolates of P. aeruginosa. In this regard, 83 strains of P. aeruginosa were collected as FQn resistance isolates from 50 burn patients. All (n=83) clinical isolates were subjected for PCR based assay. Qnr allele frequencies were found to be 3(3.6%), 9(10.8%), 0%, 5(6%) and 2(2.4%) for QnrA, QnrB, QnrC, QnrD and QnrS respectively. We found one isolate that carried both QnrA and QnrB alleles.

Current study was designed to examine the prevalence of Qnr genes in LEV resistant P. aeruginosa. Pseudomonal infections such as skin and soft tissue infections, burn and surgical wound infections are very difficult to treat because this pathogen has acquired intrinsic, adaptive as well as genetic resistance against all classes of antimicrobial drugs such as β-lactam, carbapenem, aminoglycoside and fluoroquinolones.[9]

Similar study was conducted in South China. It was reported that QnrA gene was detected in single FQnr isolate of P. aeruginosa. For this purpose, 256 isolates were tested against QnrA, QnrB, QnrC, QnrD and QnrS genes. It was reported that these genes (QnrB, QnrC, QnrD and QnrS) were not present in a single isolate of P. aeruginosa.[4]

The results of current study were in accordance with results obtained by Marjani in Iraq.[10] Presence of QnrS and QnrA gene was reported in 21% and 13.1% clinical isolate of P. aeruginosa. It was also reported that QnrS and QnrA genes were present within same isolate. In 2015, it was reported that 24% FQnr isolates of P. aeruginosa were positive for QnrS gene.[10]

Conclusion

To the best of our knowledge, this is the first report detailing prevalence of Qnr genes in a commonly encountered nosocomial pathogen such a Pseudomonas aeruginosa.Further genetic and biochemical studies are required to understand dynamics of the spread of this allele.

Recommendations

Qnr mediated resistance phenotype is generally plasmid mediated therefore highly prone to be transferred to other related pathogens through horizontal gene transfer.4 Nonetheless, such screenings are advisable on routine basis from infection control perspective.

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Conflict of Interest: None to declare.
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