A clinical debate concerning Aminoglycoside resistance genes among Pseudomonas aeruginosa strains

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Received: December 25, 2017; Accepted: January 28, 2018; Published: March 1, 2018. Citation: Pourahmad M. et al. A clinical debate concerning Aminoglycoside resistance genes among Pseudomonas aeruginosa strains. World Family Medicine. 2018; 16(3):172-176. DOI: 10.5742/MEWFM.2018.93327

Abstract

Introduction: Pseudomonas aeruginosa is a worldwide nosocomial infection that disrupts hospitalized patient's recovery. Use of the proper anti-pseudomonas antibiotic therapy is an expected challenge among health care providers.

Methods: This is a cross-sectional in-vitro study which was conducted in Isfahan in 2016-2017. After selection of the patients with P. aeroginusa infection and isolation of bacteria, the presence of AME coding genes such as aac(6')-II and ant(2'')-I was surveyed by PCR method. Result: From 350 patients with P.aeruginosa infection, 100 samples were obtained. About half of the strains were taken from urine samples and respiratory tract swap. 93% of Pseudomonas aeruginosa strains were resistant to at least one antibiotic. The highest antibiotic resistance was dedicated to Tobramycin and Meropenem. However, all strains were sensitive to Colistin. In regard to AME genome, 13 specimens had ant(2'')-I and 4 strains had aac(6')-II genes. aac(6')-II gene caused 75% resistance to Amikacin, Tobramycin, and Gentamycin while ant(2'')-I gene posed Tobramycin and Gentamycin resistance.

Conclusion: Overall, there was a high resistance ratio to various aminoglycosides, B-lactams, and fluoroquinolones isolated from infected patients. Less than 20% of strains demonstrated AME genes of aac(6')-II and ant(2'')-I.

Key words: Pseudomonas aeruginosa; Aminoglycoside; antibiotic resistance; nosocomial infection

Introduction

Nosocomial infection is a trending worldwide concern, which interrupts hospitalized patients' recovering process while posing higher mortality risk (1, 2). Microbiological studies done in both developed and developing countries claim that Pseudomonas aeruginosa is the most prevalent nosocomial strain (1, 3, 4). Pseudomonas aeruginosa is an opportunistic gram-negative bacillus which infects mostly ICU patients, burn victims, VAP (ventilator associated pneumonia), and chronic pulmonary disease patients such as those with cystic fibrosis (1, 5). Actually, it is a hospital-acquired infection, which mostly presents itself as a urinary tract infection, endocarditis, bacteremia, and sepsis (2, 4).

The real worrisome issue regarding Pseudomonas aeruginosa infection is antibiotic resistance as MDR (multi-drug resistance) increases hospitalization duration, antibiotic treatment failure, mortality, and medical expenses (6, 7). In one study in Iran, MDR Pseudomonas strains were mostly gathered from OB/GYN and ICU departments that were vulnerable to Colistin besides being resistant to B-lactam antibiotics like Piperacillin (5). Aminoglycosides are the worldwide approved treatment of choice for nosocomial Pseudomonas aeruginosa infection. However, due to chromosomal and acquired aminoglycoside resistance, B-lactam combination therapy is a wise recommendation (1, 8).

Genetic coded modifying enzymes like acetyltransferase (AAC), nucleotidyltransferase (ANT) and phosphotransferase (APH) are the most found methods that Pseudomonas aeruginosa strains are equipped with against aminoglycosides (4, 6). By reviewing previous studies, it is understood that various Pseudomonas aeruginosa MDR patterns exist based on their unique geographical location. Regular AME genome investigation is advised in order to administer proper antibiotics for P.aeruginosa infection (6, 8-10).

Hereby, this study attempts to investigate the prevalence of genetic coding of aac (6')-II and ant (2'')-I in a hospital sample of Pseudomonas aeruginosa strains and its relation to aminoglycosides' resistance.

Materials and methods

This cross-sectional in-vitro study was conducted from January 2016 to January 2017. Pseudomonas aeruginosa strains were obtained from Alzahra hospital lab, Isfahan. Patients with confirmed Pseudomonas aeruginosa culture were included in the total sample and were clinically examined.

They were from ICU, internal medicine, general surgery, infectious diseases, and orthopedic departments. We gathered information regarding gender, age, hospital department, hospitalization duration, and clinical presentation. In correlation with our inclusion criteria, all patients who had been hospitalized for more than 5 days participated in this survey. However, if the patients were previously hospitalized, had a drug history of antibiotic consumption, and low P.aeruginosa DNA purity, their samples were excluded from the final result. In order to prevent any accidental culture contamination with Pseudomonas aeruginosa, if the patients showed no clinical presentation of infectious disease such as leukocytosis, fever, and chills, their culture was removed from the final sample.

After reviewing the aforementioned inclusion and exclusion criteria, patients with clinical presentation of infectious diseases were chosen. Based on their primary diagnosis, samples of blood, CSF, IV catheter, urine, abdominal cavity fluid, sputum, wound discharge, and tracheal swab were sent for microbiological evaluation in both blood and EMB (eosin methylene blue) agars. As a matter of fact, just one sample was sent to the lab. If Pseudomonas aeruginosa strains were responsible for the clinical presentation, they produced green-pigmented colonies on EMB culture. Moreover, oxidase and movement tests were positive for Pseudomonas aeruginosa. Isolated bacilli of Pseudomonas aeruginosa were grown on a Luria Bertani (LB) agar and delivered to our Molecular Genetics Laboratory.

First, antibiotic resistance was evaluated in pseudomonas strains by disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (11), using Amikacin (30µg), Tobramycin (30µg), Gentamycin (10µg), Imipenem (10µg), Meropenem (10µg), Ciprofloxacin (5µg), Ceftazidime (30µg), Cefepime (30µg), Colistin (10µg), Piperacillin (30µg), and Piperacillin/tazobactam (100/10µg).

As well as disc diffusion stray testing, all strains were investigated in regard to aac(6')-II and ant(2'')-I genes presence by PCR (polymerase chain reaction) method. Gram-negative bacterial genomic DNA was obtained using the Kit Wizard Genomic (Promega).

The total template DNA for the PCR amplification was taken from the supernatant of a mixture of P. aeruginosa cells produced by the boiling method. PCR amplification was done using 2.5 mL of the template DNA, 1 mL of each primer, 19.5 mL master mix, and 1 mL of Taq DNA polymerase (CinnaGen) in a total volume of 25 mL.

Then, 5 ml of each PCR product was analyzed by electrophoresis on a 1% (w/v) TAE agarose gel (Fermentas UAB, Vilnius, Lithuania) containing 0.1 ml/ml ethidium bromide. The amplicons were visualized on a UV trans-illuminator and photographed. In the end, data of bacteriological antibiotic resistance, molecular-genetic identification, gender, age, hospitalized department, and hospitalization duration were gathered and analyzed by SPSS v22.

Ethics approval: This project was approved by the Ethics Committee, Deputy of Research, Isfahan University of Medical Sciences.

Results

According to our data, out of a total 350 patients only 100 Pseudomonas aeruginosa strains were obtained from patients who had clinical presentation of infectious disease during 1 year in Alzahra hospital, Isfahan. Candidates were from 13 to 83 years old while the mean age of the patients was 53±3.1 years. Out of the whole group population, 57% were male and 33% were female. The mean duration of hospitalization among patients was 63±2.7 days. Related to hospitalization, 45% of patients were admitted to surgical ward, 9% to ICU, 16% to internal medicine and infectious departments. The source of the specimens was taken from one sample: urine sample (37%), sputum and tracheal swap (21%), IV catheter (13%), blood culture (10%), CSF (7%), and tapped abdominal fluid (2%). In fact, patients admitted to ICU had P.aeruginosa infection based on strains from tracheal and IV line swap (90%). On the other hand, urinary tract infection and sepsis (83%) were highly responsible for P.aeruginosa contamination in the surgical ward.

According to the microbiological analysis, 93% of Pseudomonas aeruginosa strains were resistant to at least one antibiotic. However, 7% demonstrated no findings of disc diffusion resistance. Four patients without resistant strains were hospitalized for less than 30 days on average. All collected strains were sensitive to Colistin and 52% were susceptive to both Amikacin and Colistin.

On the other hand, 46% of strains were resistant to at least 4 antibiotics in regard to their disc diffusion findings. Furthermore, the highest resistance was detected as 76% Tobramycin followed by 73% Meropenem, 66% Imipenem,64% Cefepime,62% Ceftazidime, 49% Piperacillin, 33% Piperacillin/tazobactam, 31% Ciprofloxacin, 29% Amikacin, and 15% Gentamycin. Overall, aminoglycoside resistance was 63% among our samples (Table 1).

Table 1: Antibiotic resistance pattern among collected Pseudomonas aeruginosa strains

Antibiotic	Resistant%	Intermediate%	Sensitive%
Tobramycin	76	3	21
Meropenem	73	0	27
Imipenem	66	2	32
Ceftazidime	62	0	38
Cefepime	64	1	36
Ciprofloxacin	31	0	69
Piperacillin	49	0	51
Piperacillin/ tazobactam	33	1	67
Gentamycin	15	0	85
Colistin	0	0	100
Amikacin	29	3	68

Regarding our molecular genetic evaluation, 13 strains of Pseudomonas aeruginosa had modifying enzyme genes of ant(2'')-I and 4 strains had aac(6')-II genes. The mean age of patients with these genes was 40.5 years and 9 patients were female and 8 were male. 41.1% of the strains had been taken from a urine sample, a respiratory tract sample at 23.5%, burn wound pus 29.4%, and blood culture 7%. More than half of these patients were hospitalized in the ICU. In fact, all these specimens were completely resistant to Ciprofloxacin, Meropenem, and Imipenem and more than 90% resistant to Ceftazidime and Cefepime.

However, almost half (52.9%) were susceptible to Piperacillin/tazobactam. Strains with aac(6')-II gene showed 75% resistance to Amikacin, Gentamycin, and Tobramycin. On the other hand, Pseudomonas aeruginosa with ant(2'')-I gene revealed 100% resistance to Tobramycin and Gentamycin. Moreover, 38.4% of ant(2'')-I strains were vulnerable to Amikacin (Table 2).

Table 2: Pseudomonas aeruginosa strains with aac(6')-II and ant(2")-I and aminoglycoside resistance

Antibiotic / Resistance Gene	Amikacin%	Tobramycin%	Gentamycin%
aac (6')-II	75	75	75
ant (2'')-I	61.6	100	100

Discussion

Submitting the appropriate treatment for MDR P.aeruginosa is a breath taking and challenging assignment in the medical field. Aminoglycosides play a major role in P.aeruginosa treatment. Although combination anti-pseudomonas therapy is advised, resistance to B-lactam happens mostly through chromosomal pathway (9, 10, 12).

P.aeruginosa interrupts aminoglycosides antimicrobial effect by modifying enzymes, ceasing drug permeability, activating (MexXY-OprM) pump, activating Phop-PhoQ system, creating biofilm, and 16s rRNA methylase (5). The most common defensive mechanism among P.aeruginosa is aminoglycoside modifying enzymes (AME). Some of these enzymes are aminoglycoside phosphoryl transferase (apt), aminoglycoside nucleotidyl transferase (ant), and aminoglycoside acetyltransferase (aac) (1, 3, 4).

A study on 94 burn wounds samples found 88.3% MDR strains of P.aeruginosa which were more than 80% resistant to Ciprofloxacin and Cefepime in contrast to being vulnerable to Colistin (3). Similar to another study on 111 P.aeruginosa strains extracted of wound secretions (5), our samples of burn wounds pus were completely resistant to aminoglycosides as well as having either aac(6')-II and ant(2'')-I genes. Although all these strains were resistant against Cefepime, Ciprofloxacin, and Piperacillin/tazobactam, they were all sensitive to Colistin.

Some AME genes include aph(3')-VI, ant(2'')-I, aac(6')-II, aac(6')-I, and aac(3)-II (3, 4). Various studies claim that aac(6')-I gene causes resistance to Amikacin and Torbramycin, while aac(6')-II and ant(2'')-I interfere with Tobramycin and Gentamycin anti-pseudomonas role (1, 3, 4, 8).

Other study proves that aac(3')-I and aac(3')-II is highly responsible for resistance against Gentamycin in P.aeruginosa (6). According to another study result, aac(6')-II mostly develops resistance to Gentamycin and Tobramycin (8).

In 2011, one study has been done on 250 P.aeruginosa samples from various geographical areas in Iran in which 135 strains were resistant to aminoglycosides. The prevalence of resistant genes among their specimens were described as following: 35% aac(6')-II, 28% ant(2'')-I, and 7% aac(6')-I. Obviously, aac(6')-II and ant(2'')-I genes were the most common genome in P.aeruginosa with AME (6). Similar to the study done on MDR P.aeruginosa in Venezuela and Japan, the most common genes responsible for AME were aac(6')-II and ant(2'')-I. Also, gene aac(6')-II was known for resistance against Gentamycin (1, 4).

In regard to geographical distribution of AME genes, aac(6'')-I gene was more prevalent in Greece, France, and India and was recognized to be responsible for resistance to Amikacin. Nevertheless, aph(3')-VI and aac(6')-II genes were highly discovered in America, Korea, and Iran (1, 4). So, we focused on aac(6')-II and ant(2'')-I genes in genetic evaluation of our P.aeruginosa strains. In association to our results, only 17 strains (18.2%) had either AME genes. These specimens were collected mostly from urine samples and respiratory tract swabs. Nonetheless, ant(2'')-I gene was more prevalent among them (76.4%). P.aeruginosa strains carrying ant(2'')-I gene had full resistance to Tobramycin and Gentamycin and 61.6% resistance to Amikacin. Nonetheless, strains with acc(6')-II gene showed 75% resistance to Tobramycin, Amikacin, and Gentamycin. All strains with acc(6')-II and ant(2'')-I genes were sensitive to Colistin in contrast to being resistant to Ciprofloxacin, Cefepime, Ceftazidime, Imipenem, and Meropenem.

Conclusion

To sum up, our result indicates overall high resistance range to various aminoglycosides, B-lactams, and fluoroquinolones isolated from infected patients. In fact, less than 20% of strains demonstrated AME genes while being susceptible to Colistin. Based on our genetic evaluation, acc(6')-II gene caused equal resistance to Tobramycin, Amikacin, and Gentamycin. However, ant(2'')-I gene provided resistance to Tobramycin and Gentamycin. Therefore, we advise using accurate antibiogram and regular AME genome determination in breaking the P.aeruginosa aminoglycoside resistance vicious cycle.

Acknowledgements: This research work was financed by Isfahan University of Medical Sciences, Isfahan, Iran. This article has been from the results of the thesis of the infectious diseases specialist which is funded by Isfahan university of medical sciences.

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