Pseudomonas Aeruginosa Strains Obtained from Hospital Infections with Various Resistances In Tehran, Iran

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Objectives: Improper and inappropriate use of antibiotics is one of the possible factors affecting the transmission of antibiotic resistance is Because Pseudomonas aeruginosa is one of the most serious pathogenic bacteria in hospital environments and resistant It is an antibiotic that causes problems in treatment. This study aims to determine multiple resistances Pseudomonas has been treated with antibiotics, arsenic and metals.

Materials and Methods: In this study, 23 strains of Pseudomonas aeruginosa were isolated from clinical specimens. for review Resistance of these bacteria to penicillin antibiotics was used by Kirby-Bauer method. The minimum concentration C.I.M. (and minimum lethal concentrations (C.B.M.) of antibiotics and heavy metals (cadmium, mercury) and arsenate Tubal dilution, agar and growth were performed in agar plate, respectively.

Results: In this study, the highest and lowest MIC values obtained in Pseudomonas aeruginosa for metals, respectively. * 8 and 3 cadmiums (0.6 and 4.9 µg / ml), mercury (<0.12 and 4 µg / ml) and arsenate (10 × were 256 µg / ml). Of 23 strains, 84% to the antibiotic carbonicillin, 63% to piperacillin and 100% to 103 Arsenic and cadmium were resistant. Also, 82.6% of the strains were resistant to mercury.

Conclusion: The results of this study showed that Pseudomonas aeruginosa strains have multiple resistance to arsenic, metals and the antibiotics are car penicillin and piperacillin.

Keywords: Multiple resistance, Antibiotic, Heavy metals, Pseudomonas aeruginosa, Minimum inhibitory concentration, Minimum lethal concentration.

INTRODUCTION

Despite more than 50 years of supply and supply of penicillin, it turns out that this drug is still one of the most important and widely used anti-inflammatory drugs. Biotics are available and new penicillin kernel derivatives are produced and supplied every year. When penicillin was discovered, there was a lot of optimism about eradicating infectious diseases, but despite the remarkable success in treating diseases, the infection soon led to a new problem, and that was the emergence of penicillin resistance. This resistance leads to the selection of resistant strains among susceptible emerging species. New strains have become resistant species. In fact, the development of resistance can be due to the excessive and inappropriate use of microbial anti-inflammatory drugs in many clinical units. Even if the correct use of antimicrobial agents has positive effects on health, but resistance to these agents as a result of its incorrect use but creates additional costs. Infection with resistant pathogens causes more. mortality than susceptible pathogens. It is estimated that between $ 100 million in the United States annually resists drug agents and costs $30 billion. Today, more than 30 types of natural and semi-synthetic penicillins are produced Has been. Penicillins are based on their antimicrobial spectrum and properties Physical and chemical are divided into 5 groups. Of these Groups Two groups of carboxy penicillins and uroid and penicillin They are used to treat infections caused by Pseudomonas aeruginosa (2, 1). Because indices of resistance to penicillin and Metals such as mercury, cadmium and arsenic are common in some cases and most of their genes are located on a plasmid, therefore Evaluation of concomitant resistance to these antimicrobial agents in terms of the spread of multiple resistance in different strains of Bacteria is important and special.

Mercury derivatives such as phenyl mercuric nitrate, phenyl acetate Mercuric and thiomersal are widely used as preservatives Pharmaceutical and cosmetic fields are used, (1) purpose to do This study determined the resistance of Pseudomonas aeruginosa strains to Arstate, heavy metals and carpenicillin antibiotics and It is piperacillin, so that it can be identified as a way to fight and prevent their spread and help save patients' health appeared.

MATERIALS AND METHODS

In this study 23 strains of Pseudomonas aeruginosa were sampled Clinically isolated to determine susceptibility or resistance of Pseudomonas Antibi gram test was performed by Kirby-Bauer method to Isolation of penicillin-resistant bacteria from disc Standard antibi grams include carbenicillin and piperacillin use Took. For this purpose, first a few colonies of bacteria on the environment TSB fluid was inoculated and the tubes were inoculated at room temperature 35 was placed so that the pipes had a standard tube turbidity of 0.5 mc C ° Reach McFarland. Using a sterile swab and immerse it in the suspension Microbial and swab impregnation with suspension, suspension inoculation Microbial in three different directions on the plate surface containing the culture medium to perform a uniform inoculation (3) Store at room temperature for 3-5 minutes until the microbial liquid is absorbed Culture medium. After the above time the desired antibiotic discs Such as carbonicillin and piperacillin at intervals specified by Pence Sterile was placed on the surface of the medium. Plates for 18-16 They were placed in a greenhouse at 35 ° C. after During the above time, the plates were examined and the diameter of the halo did not grow in the area around the discs was measured with a millimeter ruler. Antibiotic-resistant specimens of the penicillin group According to the standard.

tables were determined (5 and 4) Use in stock solution of each metal is as follows. Cause Select higher concentrations in stock solutions to prepare concentrations There are different types for each material. (9-6 and 4). (L / mg (93 / 4- , Cadmium nitrate 0 / 037-0 / 075-0 / 15-0 / 31-0 / 62-1 / 23-2 / 46 -64-128 (g / l), mercury nitrate 0 / 12-0 / 25-0 / 5-1-2-4 (mg / l), Sodium Arsenate 2-4-8-16-32. Cadmium nitrate from Fluke, sodium arsenate and nitrate Mercury and microbial culture media from Merck and antibiotics were provided by Farabi Pharmaceutical Company of Isfahan. Microbial standard strains from scientific and industrial research organizations Tehran was prepared. To determine the MIC of metals after preparation of the medium II-PHG sterile agar (peptone 4 g, yeast extract 1 g, glucose 2) G and agar 15 g per 1000 ml of distilled water), during New strains have become resistant species. The temperature of the culture medium reached about 55 ° C, a metal solution with a concentration Add the specified to the culture medium and then adjust the PH. The culture media was poured into plates and allowed to stand Cool. To keep the surface of the plates free of moisture, 37 C ° was placed. To cultivate plates for 30 minutes at room temperature the desired bacteria on the culture medium can be bacterial colonies Cultivated radially on the surface of the medium or 0.1 mm Liters of microbial suspension growing in the logarithmic phase Added the surface of the culture medium and by glass rod, He spread it on the surface of the environment. Spread at ambient level. During operation of a number of plates as a sterile control Used. Plates for 24-72 hours at a temperature of 35 degrees They were placed in a greenhouse. After the above period, the plate Checked and used according to different concentrations each Metal, the amount of MIC based on the minimum concentration of that metal as it grows Bacteria inhibited and determined.

To determine the MIC of heavy metals in compatibility mode, a number Bacteria resistant to the target metal that have a high MIC Have not been selected from the bacterial colony on the culture medium II-PHG has a low concentration of cultured metal. Next, from A colony grown in the previous environment to the next II-PHG environment that has Higher concentrations of the desired metal are cultivated. this work and the concentration of the metal in the medium increases continuously for some time That the desired bacterium is not able to grow in that concentration of metal (6 and 4.) In some resistant bacteria this is an important feature Is physiologically after some time in an environment containing anti Biotics or metals found and grown will be able to Adapt to higher concentrations of those substances. in this the study tried to find that most aspects that have a higher MIC ratio Were to other strains, to be selected for compatibility mode to Their greatest compatibility with higher concentrations is evident Be. Therefore, compatibility testing for strains with lower MIC Have been, not done. Statistical analysis of the results was done by SAS software Is. For this purpose, to investigate the existence of significant differences between the results the result of the procedure models linear General and for Investigating the existence of correlations between data from correlation analysis methods Was used and $p < 0.05$ was considered significant.

RESULTS

Pseudomonas aeruginosa strains from clinical specimens of urine, ulcers, Ear and bronchial secretions were isolated and tested, most of which Pseudomonas aeruginosa strains in isolated samples from wounds Was seen (Table 1).

Table 1. Distribution of Pseudomonas aeruginosa strains based on clinical specimens isolated from it.

Type of clinical sample	Number of strains	Numeral of strains
Urine	3	(2,10,11)
Wounds	18	(3,7,8,12,13,14-23)
Ear discharge	1	(9)
Bronchu	1	(4)

From 23 strains of Pseudomonas aeruginosa, 84% were given antibiotics Carpenicillin and 63% were resistant to piperacillin. A total of 19 Strains (82.6%) were resistant to all three metals (triple resistance) And in the case of dual resistance, all strains are cadmium and arsenate Were resistant (Table 2). Minimum inhibitory concentration (MIC) (growth of Pseudomonas strains Aerogenesis in normal and adaptive states was also determined by The standard (1074 PTCC) was also used as a control and the results showed that the highest and lowest MIC values obtained in Pseudomonas aeruginosa for metals, cadmium (62% and 4.93,respectively) Micrograms per ml) of mercury (<0.12 and 4 micrograms per ml) and Arstate (103 * 8 and 103 * 256 micro-grams per ml) (Table2).

Table 2. General pattern of metal resistance in Pseudomonas aeruginosa strains isolated from clinical specimens.

Metals	Abundance Number(%)
Cadmium	(100) 23
Mercury	(82/6) 19
Arsenate	(100) 23
Cadmium and mercury	(83/6) 19
Cadmium and arsenate	(100) 23
Mercury and arsenate	(82/6) 19
Cadmium, mercury and arsenate	(82/6) 19

CONCLUSION

In the results of antibiogram testing for isolation Antibiotic-resistant bacteria of the penicillin group Most of the bacteria studied were compared to antibiotics They are durable. So that the percentage of carbon resistance Cillin and piperacillin in Pseudomonas aeruginosa strains, respectively It is equal to 84% and 63%.

In a similar review by the Race owner on the strains clinically performed Pseudomonas aeruginosa, the degree of resistance to anti the biotics of silyl, piperacillin, megacillin and ticarcillin Respectively, equal to 89, 55, 89 and 89.5 percent have been reported (10). Langaee et al. Showed the origin of this resistance the effect of expression is too much of the enzyme beta-lactamase (11) In this study on the cadmium resistance of 23 Pseudomonas strains Aeruginosa all have an MIC equal to or greater than 0.62 Micrograms per milliliter. In most strains the amount difference The MIC is so small that 14 strains have an equal MIC 2.5 µg / ml and 6 strains with MIC equal to 4.9 Micrograms per milliliter. About mercury resistance 82.6% Strains are resistant to mercury (greater than or equal to MIC) With 1 microgram per ml). All strains were resistant to arsenate (larger MIC or Equal to 4000 micrograms per milliliter). Based on the results obtained It was found that in most cases, the strains were able to grow Are in higher concentrations of the desired metal such that Their MICs in compatibility mode are very different from MICs under normal conditions has it. Silver In 1996 adaptation made a change in an organism or a population of organisms reported by it with conditions Existing environments are more adaptable. More compatibility situations in order for organisms to survive in unfavorable growing conditions and changes in gene expression may create new systems Increases bacterial survival at higher concentrations (12).

In this study, bacteria were also able to with higher concentrations of metals Used adapted and developed to reflect the findings of other researchers Approves. A total of 19 strains in Pseudomonas aeruginosa strains (82.6%). They are resistant to all three metals (triple resistance). About dual resistances of all strains simultaneously to cadmium and arsenic Resistant and 19 samples (82.61)% simultaneous resistance to They had cadmium and mercury or mercury and arsenic. In Tavakoli and Et al. MIC to cadmium and arsenic 7.5 ml, respectively Mol/L and 60 mmol/L for Pseudomonas aeruginosa It has been reported (1).

In general, the high resistance of Pseudomonas aeruginosa to metals It can be due to the nature of the cell membrane of this bacterium. Bacteria Pseudomonas aeruginosa is one of the most resistant bacteria without spores Equal to antimicrobial agents. The main reason for this resistance is the presence Different purines with other bacteria as well as the strength of the lipopoly layer Saccharide is this bacterium. So that this bacterium has It has a high inherent resistance to most antimicrobials. Resistance More than arsenic, probably due to strong diffusion systems It uses energy to remove this metal from the cell and from accumulation prevents in the cell (13 and 2).

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