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RESEARCH ARTICLE

In-vitro susceptibility of multiple drug resistant *Pseudomonas aeruginosa* to organic acids

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ABSTRACT

Objectives: *Pseudomonas aeruginosa* is a classic opportunistic pathogen with innate resistance to many antibiotics and disinfectants. Resistance to antimicrobial agents makes it the most noxious organism to eliminate from infection site. In view of its antimicrobial resistance, an attempt was made to study its susceptibility to various organic acids.

Methods: Seven clinical isolates of *P. aeruginosa* resistant to multiple antibiotics were subjected to in vitro susceptibility to various organic acids by broth dilution method to find out susceptibility to various organic acids.

Results: The isolates of *P. aeruginosa* resistant to 14 antimicrobials were found susceptible to one percent oxalic acid and trichloroacetic acid, two percent lactic acid and citric acid, and three percent acetic acid. It is interesting to note that strains resistant to multiple antibiotics were also found susceptible to organic acids. Oxalic acid and trichloroacetic acid were found highly effective.

Conclusions: Clinical use of oxalic acid, trichloroacetic acid and lactic acid as topical agents to treat superficial pseudomonal infections caused by difficult strains of *P. aeruginosa* may be recommended after confirmation of their toxicity and in vivo efficacy in animal models. *J Microbiol Infect Dis 2013; 3(2): 67-70*

Key words: *Pseudomonas aeruginosa*, Multiple Antibiotic Resistance, Susceptibility to Organic Acids.

Çoklu ilaç dirençli Pseudomonas aeruginosa kökenlerinin organik asitlere invitro duyarlılıkları

ÖZET

Amaç: *Pseudomonas aeruginosa* fırsatçı bir patojen olup birçok antibiyotiğe ve dezenfektana doğal dirençlidir. Antimikrobiyal direnci nedeniyle bu etkenleri elimine etmek zordur. Antimikrobiyal direnci göz önüne alınarak bakterilerin çeşitli organik asitlere duyarlılığının araştırılması amaçlanmıştır.

Yöntemler: Toplam yedi dirençli *P. aeruginosa* izolatının çeşitli organik asitlere karşı etkinliği sıvı mikrodilüsyon tekniği ile araştırıldı.

Bulgular: Farklı 14 antibiyotiğe dirençli *P. aeruginosa* izolatının %1 oranında oksalik asit ve trikloroasetik asite, %2 laktik asit ve sitrik aside %3 oranında asetik asite hassas olduğu gözlendi. İlginç olan nokta ise birçok antibiyotiğe dirençli olan bu kökenler organik asitlere hassastı. Oksalik asid ve trikloroasetik asit oldukça etkin bulundu.

Sonuç: Bulgularımız toksisite ve invivo etkinlik testleri ile doğrulanmasının ardından oksalik asit, trikloro asetik asit ve laktik asit tedavisi güç *P. aeruginosa* enfeksiyonlarının topikal tedavisinde kullanılması önerilebilir.

Anahtar kelimeler: *Pseudomonas aeruginosa*, çoklu antibiyotik direnci, organik asidlere duyarlılık.

INTRODUCTION

Pseudomonas aeruginosa is a classic opportunistic pathogen with innate resistance to many antibiotics and disinfectants. It is ubiquitous in the hospital environment and because of its ability to survive in the hospital environment; it creates threat to patient care. It is the most difficult nosocomial pathogen to be eliminated from infection site. In spite of availability of newer antimicrobial agents with broad spectrum of activity, it is difficult to treat wound infections caused by nosocomial strains of *P. aeruginosa*. A

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variety of chemical agents are available, which are nontoxic, inexpensive and highly effective against nosocomial strains of *P. aeruginosa*. It has been reported that in some cases of local applications, chemical agents have advantages over antibiotics, especially in controlling hospital strains of *P. aeruginosa*, which are resistant to multiple antipseudomonal agents commonly used in the treatment of pseudomonal wound infections.[1] These agents can be used locally in the treatment of pseudomonal wound infections and the use of antibiotics can be avoided to some extent. *P. aeruginosa* possesses a considerable degree of natural resistance to antibiotics. Antibiotic treatment is not always satisfactory in the management of Pseudomonal infections.[2] Although, *P. aeruginosa* is resistant to multiple antibiotics, it is sensitive to acid media and is unable to grow at below pH 5.5.[3-4] The use of various organic acids notably acetic acid and citric acid for elimination of *P. aeruginosa* from skin and soft tissue infections and from burn infections has been reported by various workers.[5-10]

In the present study we aimed to investigate in vitro sensitivity of *P. aeruginosa* to various organic acids such as oxalic acid, lactic acid and trichloroacetic acid in addition to acetic acid and citric acid.

METHODS

For this study, seven isolates of *P. aeruginosa* isolated and identified by using conventional methods[11] from patients suffering from pseudomonal wound infections. The antimicrobial susceptibility of these isolates was studied by Kirby-Bauer disc diffusion method[12] using fifteen different antimicrobial agents. To determine susceptibility to various organic acids, research grade or analytical grade of organic acids were obtained from Hi-Media laboratories Mumbai and Qualigens, Mumbai and five different concentrations of various acids (acetic acid, citric acid, oxalic acid, lactic acid and trichloroacetic acid) were tested against seven isolates of *P. aeruginosa* from patients suffering from superficial pseudomonal infections.

To determine the minimum inhibitory concentration (MIC) of various acids, each isolate of *P. aeruginosa* was inoculated into peptone water and incubated for four hours. After incubation, turbidity of peptone water was matched with Mc Farland standard (No.1) and adjusted accordingly.[13] A set of five test tubes for each acid was taken to study the effect of five different concentrations. A 100 µL of peptone water culture was taken in five tubes and 100 µL of five different concentrations were added to five different tubes. After proper mixing, subcultures were made on pseudomonas isolation agar (PIA) after 5, 10 and 15 minutes of exposure. The PIA plates were incubated at 37˚C and after overnight incubation plates were observed for growth. The test was repeated again to confirm the reproducibility of results.

RESULTS

Table 1 shows resistance pattern of clinical isolates of *P. aeruginosa*. These isolates were found resistant to 10 to 14 antimicrobial agents. All isolates were found susceptible to ceftazidime (100%) and six isolates were susceptible to amikacin (85.7%). No isolates were found susceptible to the fluoroquinolones group of antimicrobial agents and to netillin, ticarcillin, cephotaxime and ceftriaxone.

Table 1. Antimicrobial Resistance and Susceptibility Pattern of Isolates of *P. aeruginosa*.

Isolate No	Resistance Pattern	Susceptibility Pattern
1	Cb, Ce, Cf, Ci, Cs, G, Nt, Nx, Of, Pc, Pi, Tb, Ti	Ak, Ca
2	Ce, Cf, Ci, Cs, Nt, Nx, Of, Pc, Pf, Tb, Ti	Ca
3	Ce, Cf, Ci, Cs, Nt, Nx, Of, Pc, Pf, Ti	Ak, Ca
4	Ak, Cb, Ce, Cf, Ci, Cs, G, Nt, Nx Of, Pc, Pf, Tb, Ti	Ak, Ca, Cs, Tb
5	Cb, Ce, Cf, Ci, Cs, G, Nt, Nx, Of, Pc, Pf, Tb, Ti	Ak, Ca, Cb, Pc
6	Cb, Ce, Cf, Ci, G, Nt, Nx, Of, Pc, Pf, Ti	Ak, Ca, Cb, G, Tb
7	Ce, Cf, Ci, Cs, G, Nt, Nx, Of, Pf, Tb, Ti	Ak, Ca, Cb, G

Ak=amikacin, Ci=ceftriaxone, Of=ofloxacin, Ca=ceftazidime, Cs=cefoperazone, Pc=piperacillin, Cb-carbenicillin, G=gentamicin, Pf=pefloxacin, Ce=cephotaxime, Nt=netillin, Tb=tobramycin, Cf=ciprofloxacin, Nx=norfloxacin, Ti=ticarcillin

Table 2 shows susceptibility pattern of *P. aeruginosa* to various acids. All seven isolates were found to be inhibited by one percent oxalic acid and trichloroacetic acid, two percent citric acid and lactic acid, and three percent acetic acid. *P. aeruginosa* was also found to be inhibited by one percent lactic acid when exposed for 10 minutes or more. Six isolates were inhibited by one percent citric acid and three isolates were inhibited by two percent acetic acid when exposed for 15 minutes.

Table 2. In vitro Effects of Various Organic Acids against *P. aeruginosa*

Exposure time		Number of isolates inhibited at different concentrations of organic acids (N-7)				
Minutes	Name of Acid	1%	2%	3%	4%	5%
05 Min	Acetic Acid	00	00	07	07	07
10 Min	Acetic Acid	00	00	07	07	07
15 Min	Acetic Acid	00	03	07	07	07
05 Min	Citric Acid	00	07	07	07	07
10 Min	Citric Acid	03	07	07	07	07
15 Min	Citric Acid	06	07	07	07	07
05 Min	Oxalic acid	07	07	07	07	07
10 Min	Oxalic acid	07	07	07	07	07
15 Min	Oxalic acid	07	07	07	07	07
05 Min	Lactic Acid	02	07	07	07	07
10 Min	Lactic Acid	07	07	07	07	07
15 Min	Lactic Acid	07	07	07	07	07
05 Min	Trichloroacetic Acid	07	07	07	07	07
10 Min	Trichloroacetic Acid	07	07	07	07	07
15 Min	Trichloroacetic Acid	07	07	07	07	07

DISCUSSION

Pseudomonas infection has always been a problem to the clinicians. Traditional therapies with aminoglycosides or antipseudomonal penicillins have their own limitations. An infection by multiple antibiotic resistant *P. aeruginosa* seriously hampers the therapy and needs special attention. Its resistance many currently available antipseudomonal agents have been reported, especially the nosocomial strains are highly resistant to various antibiotics. In the present study also, the isolates of *P. aeruginosa* were found resistant to 10-14 different antimicrobial agents from different groups including fluoroquinolones, aminoglycosides and third generation cephalosporins.

In the present study, all seven isolates were found to be inhibited by all organic acids used. Thrichloroacetic acid and oxalic acid were found most effective followed by lactic acid, citric acid and acetic acid. Susceptibility to acetic acid and citric acid and their use in the treatment of superficial nosocomial infections caused by *P. aeruginosa* has been previously reported.[5-10]

The topical use of various acids for elimination of *P. aeruginosa* from skin and soft tissue infections and from burn infections has been reported by various workers.[5-8] The use of acetic acid has been reported from time to time as a topical agent for the treatment of pseudomonal infections of burns and skin and soft tissue infections. In a study conducted by Sloss et al. all strains of *P. aeruginosa* exhibited a minimum inhibitory concentration of 2% acetic acid and application of 2% acetic acid to wounds resulted in elimination of *P. aeruginosa* from the wounds of 14 of the 16 patients within two weeks of treatment.[5] Al-Ibran and Khan found that application of 1% acetic acid to burns wounds cleared *P. aeruginosa* in 19% cases.[6] However, Juma et al. reported to 2% acetic acid effective against *P. aeruginosa* in vitro.[7] in our previous study, *P. aeruginosa* was found to be inhibited by 3% acetic acid in in vitro study.[8] Thus, acetic acid was shown to be an

inexpensive and efficient agent for the elimination of multiple antibiotic resistant strains *P. aeruginosa* from burn and soft tissue wounds in a concentration of 1-5%. The findings of the present study are in agreement with the earlier reports.

Although some of the strains of *P. aeruginosa* were in habited by 1% citric acid in in vitro study, all the strains of *P. aeruginosa* were found to be inhibited by 2% citric acid. This findings is similar to our earlier reports in which 2% citric acid has been reported to be effective in in vitro studies.[9-10]

In the present study, oxalic acid, lactic acid and trichloroacetic acid were also found highly effective in in vitro study. To the best of our knowledge, the activity of these acids against *P. aeruginosa* has not been reported so far. Oxalic acid and trichloroacetic acid were found most effective agents than other acids. The antimicrobial activity of various organic acids may be attributed to acidic environment created by them, which makes an environment unsuitable for growth and multiplication of *P. aeruginosa* as *P. aeruginosa* unable to grow at pH below 5.5.3

CONCLUSIONS

These organic acids (oxalic acid, lactic acid and trichloroacetic acid) can be used clinically as more effective topical agents than acetic acid and citric acid for the treatment of skin and soft tissue infections, and burns infections caused by multiple antibiotic resistant strains of *P. aeruginosa*. The limitation of this study is lack of in vivo assessment of activity of oxalic acid, lactic acid and trichloroacetic acid, which were found more effective against *P. aeruginosa* in vitro studies than acetic acid and citric acid and also the study of their toxicity, adverse effects and their in vivo efficacy. Further studies regarding their toxicity, adverse effects and their in vivo efficacy using animal models will help to achieve more useful and concrete conclusion.

Conflict of Interest: None to declare

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