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STUDY ON EFFECT OF PERMEABILIZERS ON POTENTIATION OF DIFFERENT ANTIBIOTICS AGAINST DRUG RESISTANT PSEUDOMONAS AERUGINOSA ISOLATES

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ABSTRACT

Multiple drug resistant *Pseudomonas aeruginosa* is highly prevalent nosocomial pathogen responsible for unsuccessful of treatment therapies during various life threatening infections. In the current research work, effect of different concentrations of permeabilizers i.e. Citric acid, EDTA and Tris was studied against 57 clinical isolates of multiple drug resistant *Pseudomonas aeruginosa*. Significant potentiation was observed with Citric acid and EDTA while Tris does not show any sign of potentiation with different antibiotics used in the current study.

KEYWORDS: Permeabilizers, Antibiotics, *Pseudomonas aeruginosa*, Resistant.

INTRODUCTION

Pseudomonas aeruginosa, an increasingly prevalent opportunistic human pathogen, is the most common gram-negative bacterium responsible for nosocomial infections especially in patients with a compromised immunological function. *Pseudomonas aeruginosa* is responsible for producing a broad range of clinical infections including urinary tract infections, pneumonia, meningitis, surgical wounds and burn infections. Patients with Cystic fibrosis are also susceptible to colonization by the mucoid strains.[1,2] Treatment of the infections produced by *Pseusomonas aeruginosa* is difficult because of resistance of this microorganism to most of the antibiotics commonly used in human therapy, including broad-spectrum antibiotics. One of the causes of this resistance in gram-negative bacteria is the so-called intrinsic resistance, which is thought to be related to the limited capacity of many

antibiotics to diffuse through the outer membrane (OM) which thus cannot reach their target.[3-6]

In order to abridge the treatment against MDR *Pseudomonas aeruginosa* strains, different permeabilizing agents have been investigated as potentiator of various antimicrobial agents.

MATERIALS AND METHODS

The present study was conducted at Medical microbiology laboratory of Postgraduate Teaching Department of Microbiology, Rasthrasant Tukadoji Maharaj Nagpur University, Nagpur. A total of 110 clinical isolates were purified and characterized as *Pseudomonas aeruginosa* from different samples including Pus, Wound swab, Sputum, Ear swab and Urine.[7,8] The antimicrobial susceptibility testing of the isolates was carried out by disc diffusion technique using Mueller-Hinton agar plates according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[9] Uniform lawn of the isolates was made and antibiotic discs were placed on Mueller-Hinton agar plates, incubated at 37^{o}C for 18-24 hrs and the diameter of inhibition zone was measured in millimeters. Following antibiotic discs (Himedia) were used: Amikacin 30µg, Aztreonam 30µg, Ceftazidime 30µg, Ciprofloxacin 5µg, Colistin 10µg, Cefipime 30µg, Gentamicin 10µg, Meropenem 10µg, Netilmicin 30µg, Piperacillin/Tazobactam 100/10µg, Tobramycin 10µg. Depending on the antibiotic resistance pattern obtained only 57 clinical isolates were selected randomly for the further study. In the present investigation Citric acid, Ethylenediamine tetra-acetic-acid (EDTA) and Tris were used as permeabilizers. To study the potentiation effect of permeabilizers, same procedure was followed as in antibiotic susceptibility test except different concentrations of permeabilizers [Citric acid (0.05%, 0.1% and 0.15%), EDTA (0.5 mM, 1 mM and 1.5 mM), Tris (25mM, 50mM and 75mM)] were added in the medium (MHA) and respective antibiotic discs were placed after uniform inoculation of the organism and the results were observed.

RESULTS

Table 1 represents effect of Citric acid on multidrug resistant *Pseudomonas aeruginosa* (n=57) isolated from various clinical samples. *Pseudomonas aeruginosa* isolates showed maximum resistance against Tobramycin (92.98%) and Gentamicin (91.23%) among the aminoglycoside antibiotics. Considerable resistance was also observed against Amikacin (61.40%) and Netilmicin (54.39%). Resistance against some other antibiotics was more than 50% as in case of Ciprofloxacin (70.18%), Aztreonam (66.67%) and Ceftazidime (57.89%).

Out of 57 *Pseudomonas aeruginosa* isolates 28 (49.12%) were resistant to Meropenem, while 26 (45.61%) isolates showed resistance to Piperacillin/Tazobactam and 22 (38.60%) isolates were resistant to Cefepime. *Pseudomonas aeruginosa* isolates showed highest susceptibility to Colistin (94.74%). When Citric acid (0.05%) was added along with antibiotics, negligible effect was observed with Aztreonam (59.65%) and Ceftazidime (54.39%) while no changes in resistance pattern were observed with other antibiotics.

But when Citric acid (0.1%) was added significant changes were observed in resistance pattern of *Pseudomonas aeruginosa* isolates. For Amikacin and Cefepime the resistance rate became 0%, while for some of antibiotics like Ceftazidime, Ciprofloxacin and Gentamicin the resistance rate drops below 10%. Significant decrease in resistance was also observed against Meropenem (10.53%), Tobramycin (14.04%), Piperacillin/Tazobactam (24.56%) and Netilmicin (35.09%). Further when concentration of Citric acid was increased to 0.15% no further reduction in resistance was noted.

Table 2 represents effect of EDTA on multidrug resistant *Pseudomonas aeruginosa* (n=57) isolated from various clinical samples. *Pseudomonas aeruginosa* showed highest resistance against Tobramycin (92.98%) and Gentamicin (91.23%) while for some antibiotics the resistance was more than 50%. When antibiotic susceptibility testing was done with EDTA (0.5mM), the resistance against Aztreonam, Cefepime, Ceftazidime, Ciprofloxacin and Piperacillin/Tazobactam reduced below 50% as compared when antibiotics were used alone. For Aminoglycoside antibiotics resistance reduced considerably with EDTA (0.5mM). As for Amikacin it reduced from 61.40% to 47.37%, Gentamicin from 91.23% to 70.18%, Netilmicin from 54.39% to 31.58% and for Tobramycin it reduced from 92.98% to 49.12%. But when EDTA (1.0mM) was used, overall decrease in resistance was observed for most of the antibiotics. In case of Aztreonam the resistant rate was reduced from 33.33% to 0%. More significantly in case of Tobramycin the resistance was reduced from 49.12% to 14.04% and for Gentamicin it was reduced from 70.18% to 12.28%. For Amikacin and Netilmicin the resistance was reduced from 47.37% to 5.26% and 31.58% to 1.75% respectively. Considering the resistance with respect to Meropenem and Piperacillin/Tazobactam, the reduction observed was 3.51% and 1.75% respectively. While, in case of both the Cephalosporins, resistance rate was reduced below 10%. No significant changes were observed with EDTA (1.5mM).

Table 3 represents effect of Tris on multidrug resistant *Pseudomonas aeruginosa* (n=57) isolated from various clinical samples. It is observed that Tris 25mM does not have any effect in the change of resistant pattern. However, when Tris 50mM was added, very few changes in resistant pattern were observed. Amongst Aminoglycoside antibiotics highest potentiation was observed for Gentamicin as the resistance was lowered from 91.23% to 80.70%, followed by Tobramycin from 92.98% to 84.21%. Potentiation effect for Amikacin and Netilmicin was comparatively lesser with Tris 50mM. Other antibiotics showing little potentiation effect includes Ceftazidime, Piperacillin/Tazobactam, Ciprofloxacin and Cefepime. Tris 75mM showed negligible changes in resistance pattern with respect to Ciprofloxacin and Gentamicin, while no effect was observed on other antibiotics at all. Considering Aztreonam, Colistin and Meropenem, no changes in resistant pattern were observed with different concentrations of Tris.

Table. 1: Effect of Citric acid on AST pattern of selected MDR *P. aeruginosa* clinical isolates (n=57).

Sr. No	Antibiotics	Antibiotic alone (control)			Citric acid 0.05%			Citric acid 0.1%			Citric acid 0.15%		
		S	I	R	S	I	R	S	I	R	S	I	R
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
1	Amikacin	22 (38.60)	0 (00)	35 (61.40)	22 (38.60)	0 (00)	35 (61.40)	56 (98.24)	1 (1.75)	0 (00)	56 (98.24)	1 (1.75)	0 (00)
2	Aztreonam	19 (33.33)	0 (00)	38 (66.67)	19 (33.33)	04 (7.01)	34 (59.65)	24 (42.10)	19 (33.33)	14 (24.56)	25 (43.86)	18 (31.58)	14 (24.56)
3	Cefepime	35 (61.40)	0 (00)	22 (38.60)	35 (61.40)	0 (00)	22 (38.60)	50 (87.72)	7 (12.28)	0 (00)	50 (87.72)	7 (12.28)	0 (00)
4	Ceftazidime	24 (42.10)	0 (00)	33 (57.89)	24 (42.10)	02 (3.51)	31 (54.39)	45 (78.95)	8 (14.04)	4 (7.01)	45 (78.95)	8 (14.04)	4 (7.01)
5	Ciprofloxacin	10 (17.54)	7 (12.28)	40 (70.18)	10 (17.54)	7 (12.28)	40 (70.18)	35 (61.40)	20 (35.09)	2 (3.51)	35 (61.40)	20 (35.09)	2 (3.51)
6	Colistin	54 (94.74)	0 (00)	3 (5.26)	54 (94.74)	0 (00)	3 (5.26)	56 (98.24)	0 (00)	1 (1.75)	56 (98.24)	0 (00)	1 (1.75)
7	Gentamicin	2 (3.51)	3 (5.26)	52 (91.23)	2 (3.51)	3 (5.26)	52 (91.23)	39 (68.42)	14 (24.56)	4 (7.01)	39 (68.42)	14 (24.56)	4 (7.01)
8	Meropenem	27 (47.37)	2 (3.51)	28 (49.12)	27 (47.37)	2 (3.51)	28 (49.12)	42 (73.68)	9 (15.79)	6 (10.53)	42 (73.68)	9 (15.79)	6 (10.53)
9	Netilmicin	26 (45.61)	0 (00)	31 (54.39)	26 (45.61)	0 (00)	31 (54.39)	27 (47.37)	10 (17.54)	20 (35.09)	27 (47.37)	10 (17.54)	20 (35.09)
10	Piperacillin/Tazobactam	28 (49.12)	3 (5.26)	26 (45.61)	28 (49.12)	3 (5.26)	26 (45.61)	31 (54.39)	12 (21.05)	14 (24.56)	31 (54.39)	12 (21.05)	14 (24.56)
11	Tobramycin	4 (7.01)	0 (00)	53 (92.98)	4 (7.01)	0 (00)	53 (92.98)	32 (56.14)	17 (29.82)	8 (14.04)	32 (56.14)	18 (31.58)	7 (12.28)

***S:** Sensitive, **I:** Intermediate, **R:** Resistant.*

Table. 2: Effect of EDTA on AST pattern of selected MDR *P. aeruginosa* clinical isolates (n=57).

Sr. No	Antibiotics	Antibiotic alone (control)			EDTA 0.5mM			EDTA 1.0mM			EDTA 1.5mM		
		S	I	R	S	I	R	S	I	R	S	I	R
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
1	Amikacin	22 (38.60)	0 (00)	35 (61.40)	24 (42.10)	6 (10.52)	27 (47.37)	49 (85.96)	5 (8.77)	3 (5.26)	49 (85.96)	5 (8.77)	3 (5.26)
2	Aztreonam	19 (33.33)	0 (00)	38 (66.67)	19 (33.33)	19 (33.33)	19 (33.33)	45 (78.95)	12 (21.05)	0 (00)	45 (78.95)	12 (21.05)	0 (00)
3	Cefepime	35 (61.40)	0 (00)	22 (38.60)	36 (63.16)	12 (21.05)	9 (15.79)	50 (87.72)	2 (3.51)	5 (8.77)	50 (87.72)	2 (3.51)	5 (8.77)
4	Ceftazidime	24 (42.10)	0 (00)	33 (57.89)	37 (64.92)	10 (17.54)	10 (17.54)	48 (84.21)	6 (10.52)	3 (5.26)	48 (84.21)	6 (10.52)	3 (5.26)
5	Ciprofloxacin	10 (17.54)	7 (12.28)	40 (70.18)	14 (24.56)	25 (43.86)	18 (31.58)	33 (57.89)	18 (31.58)	6 (10.52)	33 (57.89)	18 (31.58)	6 (10.52)
6	Colistin	54 (94.74)	0 (00)	3 (5.26)	54 (94.74)	0 (00)	3 (5.26)	57 (100)	0 (00)	0 (00)	57 (100)	0 (00)	0 (00)
7	Gentamicin	2 (3.51)	3 (5.26)	52 (91.23)	5 (8.77)	12 (21.05)	40 (70.18)	34 (59.65)	16 (28.07)	7 (12.28)	34 (59.65)	16 (28.07)	7 (12.28)
8	Meropenem	27 (47.37)	2 (3.51)	28 (49.12)	29 (50.88)	8 (14.04)	20 (35.09)	46 (80.70)	9 (15.79)	2 (3.51)	46 (80.70)	9 (15.79)	2 (3.51)
9	Netilmicin	26 (45.61)	0 (00)	31 (54.39)	27 (47.37)	12 (21.05)	18 (31.58)	49 (85.96)	7 (12.28)	1 (1.75)	49 (85.96)	7 (12.28)	1 (1.75)
10	Piperacillin/Tazobactam	28 (49.12)	3 (5.26)	26 (45.61)	29 (50.88)	21 (36.84)	7 (12.28)	47 (82.46)	9 (15.79)	1 (1.75)	47 (82.46)	9 (15.79)	1 (1.75)
11	Tobramycin	4 (7.01)	0 (00)	53 (92.98)	8 (14.04)	21 (36.84)	28 (49.12)	44 (77.19)	5 (8.77)	8 (14.04)	44 (77.19)	5 (8.77)	8 (14.04)

***S:** Sensitive, **I:** Intermediate, **R:** Resistant.*

Table. 3: Effect of Tris on AST pattern of selected MDR *P. aeruginosa* clinical isolates (n=57).

Sr. No	Antibiotics	Antibiotic alone (control)			Tris 25mM			Tris 50mM			Tris 75mM		
		S	I	R	S	I	R	S	I	R	S	I	R
		No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
1	Amikacin	22 (38.60)	0 (00)	35 (61.40)	22 (38.60)	0 (00)	35 (61.40)	22 (38.60)	1 (1.75)	34 (59.65)	22 (38.60)	01 (1.75)	34 (59.65)
2	Aztreonam	19 (33.33)	0 (00)	38 (66.67)	19 (33.33)	0 (00)	38 (66.67)	19 (33.33)	0 (00)	38 (66.67)	19 (33.33)	0 (00)	38 (66.67)
3	Cefepime	35 (61.40)	0 (00)	22 (38.60)	35 (61.40)	0 (00)	22 (38.60)	36 (63.16)	0 (00)	21 (36.84)	36 (63.16)	0 (00)	21 (36.84)
4	Ceftazidime	24 (42.10)	0 (00)	33 (57.89)	24 (42.10)	0 (00)	33 (57.89)	28 (49.12)	4 (7.02)	25 (43.86)	28 (49.12)	4 (7.02)	25 (43.86)
5	Ciprofloxacin	10 (17.54)	7 (12.28)	40 (70.18)	10 (17.54)	7 (12.28)	40 (70.18)	11 (19.30)	7 (12.28)	39 (68.42)	12 (21.05)	7 (12.28)	38 (66.67)
6	Colistin	54 (94.74)	0 (00)	3 (5.26)	54 (94.74)	0 (00)	3 (5.26)	54 (94.74)	0 (00)	3 (5.26)	54 (94.74)	0 (00)	3 (5.26)
7	Gentamicin	2 (3.51)	3 (5.26)	52 (91.23)	2 (3.51)	3 (5.26)	52 (91.23)	4 (7.02)	7 (12.28)	46 (80.70)	4 (7.02)	8 (14.03)	45 (78.95)
8	Meropenem	27 (47.37)	2 (3.51)	28 (49.12)	27 (47.37)	2 (3.51)	28 (49.12)	27 (47.37)	2 (3.51)	28 (49.12)	27 (47.37)	2 (3.51)	28 (49.12)
9	Netilmicin	26 (45.61)	0 (00)	31 (54.39)	26 (45.61)	0 (00)	31 (54.39)	26 (45.61)	4 (7.02)	27 (47.37)	26 (45.61)	4 (7.02)	27 (47.37)
10	Piperacillin/Tazobactam	28 (49.12)	3 (5.26)	26 (45.62)	28 (49.12)	3 (5.26)	26 (45.62)	28 (49.12)	6 (10.53)	23 (40.35)	28 (49.12)	6 (10.53)	23 (40.35)
11	Tobramycin	4 (7.02)	0 (00)	53 (92.98)	4 (7.02)	0 (00)	53 (92.98)	4 (7.02)	5 (8.77)	48 (84.21)	4 (7.02)	5 (8.77)	48 (84.21)

***S:** Sensitive, **I:** Intermediate, **R:** Resistant.*

DISCUSSION

In the current research work, significant potentiation of different antibiotics was shown by Citric acid at 0.1% concentration against drug resistant *P. aeruginosa* clinical isolates and highest potentiation was observed for Aminoglycoside antibiotics except Netilmicin. Citric acid (0.1%) showed marked potentiation of Ciprofloxacin as resistance rate was lowered from 70.18% to 3.51%. Correlation was observed with the study which showed two fold decreased in MIC of Ciprofloxacin with Citric acid against *Pseudomonas aeruginosa*.[10] Reports have also been published showing Citric acid as potential permeabilizer against drug resistant *Pseudomonas aeruginosa* and *E. coli* respectively.[11,12]

Significant potentiation of various antibiotics has also been observed with EDTA (1.0mM) including Amikacin, Aztreonam, Ciprofloxacin, Meropenem, Piperacillin/Tazobactam and Tobramycin against drug resistant *Pseudomonas aeruginosa*. With Fluoroquinolone antibiotic i.e. Ciprofloxacin, EDTA (1.0mM) resulted in the increase in sensitivity rate up to three times. Similar results were observed in studies showing considerable reduction in MIC of Ciprofloxacin with EDTA against *P. aeruginosa*.[10,13] Studies have also been published on effect of antibiotic-EDTA combination on Rabbits skin artificial ulcer(s) infected by *Pseudomonas aeruginosa* and showed significant improvement in healing of artificial ulcer(s) suggesting the use of proper amount of EDTA with antibiotics to control *Pseudomonas aeruginosa*.[14]

From results represented in **Table 2** it was found that EDTA had remarkable potentiation effect on Aminoglycoside antibiotics and these results are in agreement with a study showing synergistic effect of EDTA on Amikacin and Gentamicin against *Pseudomonas aeruginosa*.[13] Effect of EDTA along with antibiotics against prominent pathogens have also been studied[15] and reported positive results of such combination on *Pseudomonas aeruginosa* which showed increase in sensitivity of Ciprofloxacin and Gentamicin and the results were in agreement with the findings of current research work. Tris does not show much antibiotic potentiation in comparison with Citric acid and EDTA against *Pseudomonas aeruginosa*. However, no literature is available for Tris alone as potentiating agent, but various studies have been reported for Tris with EDTA which showed reasonable enhancement in potentiation of various antibiotics against variety of pathogenic Organisms.[16-22]

CONCLUSION

Multiple drug resistance is a paramount challenge globally for the efficient treatment and foremost public health problem amongst the developing countries like India. Use of permeabilizers like Citric acid and EDTA along with antibiotic drugs, definitely lend a hand in lowering this global issue of antibiotic resistance in microorganisms.

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