Scientific Research Publishing

Advances in Microbiology, 2019, 9, 877-892
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Effect of Combined Therapy on Colistin Resistant *Pseudomonas aeruginosa*: An *in Vitro* Study

Nuzhath Fatima[1*], Amany Marwan[1], Alkhansa Anwar[1], Aymen Madhkhali[2], Nahed Ali[1], Areej Gadi[1], Amany Hasnany[1], Razan Hassan Hamali[1], Mousa Ali Ayyashi[3], Yahya Hobani[2]

[1]Department of Medical Laboratory Technology, College of Applied Medical Sciences, Jazan University, Jazan, KSA
[2]College of Applied Medical Sciences, Jazan University, Jazan, KSA
[3]Prince Mohammed Bin Nasser Hospital, Jazan, KSA
Email: *nfatima@jazanu.edu.sa

How to cite this paper: Fatima, N., Marwan, A., Anwar, A., Madhkhali, A., Ali, N., Gadi, A., Hasnany, A., Hamali, R.H., Ayyashi, M.A. and Hobani, Y. (2019) Effect of Combined Therapy on Colistin Resistant *Pseudomonas aeruginosa*: An *in Vitro* Study. *Advances in Microbiology*, **9**, 877-892.
https://doi.org/10.4236/aim.2019.910054

Received: September 16, 2019
Accepted: October 18, 2019
Published: October 21, 2019

Abstract

Pseudomonas aeruginosa is known for its antibiotic resistance to the clinicians. The infections caused by this pathogen are hard to treat because of its highly versatile property to mutate and acquire drug resistance. *Pseudomonas aeruginosa* also possesses intrinsic property of resistance to certain antibiotics like tetracyclines. However; in a practice to overcome the problem of multi drug resistance, clinicians restored the use of some antibiotics that were previously been used to treat the Pseudomonal infections; but they were discontinued because of its toxic effects. Colistin is an example of one such antibiotic. Use of Colistin was barred for its neurotoxicity. However in recent clinical trials, Colistin was reintroduced to fight with this superbug. Sadly in recent years, *Pseudomonas aeruginosa* developed resistance to Colistin as well. Therefore combined therapy is an alternate and suitable treatment to overcome the infections caused by multidrug-resistant *Pseudomonas aeruginosa.* The present study is an *in vitro* study; in which we tested synergy between two antibiotics namely streptomycin and Colistin on 29 clinical isolates of *P. aeruginosa* collected from hospitals in Jazan city KSA. The combination of two drugs showed synergistic activity on 55.1% of tested strains, while 20.6% strains had partial synergy, whereas indifferent synergy was observed in 13.8% strains and the 6.8% of strains had additive synergy. In addition to this, the drugs when combined also showed antagonism on one strain (3.44%). The present study showed synergistic action on Colistin-resistant *Pseudomonas aeruginosa* to greater extent (55.1%) by the two tested drugs. Hence Colistin and streptomycin can be used as a suitable combination therapy (*in vivo*) to treat multidrug resistant *P. aeruginosa* infections.

Keywords

"MDR"—Multidrug Resistant, "MIC"—Minimum Inhibitory Concentration, "*FICI*"—Fractional Inhibitory Concentration Index, "MHB"—Muller Hinton Broth, *Pseudomonas aeruginosa*

1. Introduction

Antibiotic resistance is one of the major concerns in treating the bacterial infections around the world; *Pseudomonas aeruginosa* is among the fast-developing antibiotic-resistant bacterial species; this bacterium is a gram-negative, opportunistic pathogen and a significant cause of acute and chronic infections in patients with compromised host defenses. *Pseudomonas aeruginosa* is a leading cause of nosocomial infections, such as infections of urinary tract, surgical sites wounds, bacteremia and middle ear infections etc. [1]. This organism is highly susceptible to mutations and is continuously exhibiting resistance to a number of antibiotics. Standard antibiotic regimes against *P. aeruginosa* are increasingly becoming ineffective due to the rise in drug resistance [2]. The treatment of the infections caused by this pathogen is getting difficult day by day because of the emergence of (MDR) multi drug resistance strains [3] [4]. *P. aeruginosa* is popular for its antibiotic resistance against drugs such as carbapenems, fluoroquinolones and aminoglycosides [3]. Mechanisms responsible for multidrug resistance include restricted permeability to drugs, changes in efflux systems; drug inactivation and changes in targets [5]. Apart from being resistant to antibiotics the sub-inhibitory concentrations of antibiotics such as tetracyclines can increase the cytotoxicity of this bacteria by four folds; in addition to this, sub-inhibitory concentrations of antibiotics namely tobramycin and ciprofloxacin induce biofilm formation in *Pseudomonas aeruginosa* [6]. Many countries have reported the rise of antibiotic-resistant strains of *P. aeroginosa*; Among Gulf co-operation council (GCC) countries, 92.3% clinical isolates of *P. aeroginosa* from Saudi Arabia were reported to be resistant to antibiotics [7]. In order to fight with multidrug-resistant Pseudomonal infections Colistin is the drug of choice. Many studies have communicated Colistin treatment being successful; in patients with nosocomial infections, pulmonary infections and in cancer patients suffering from MDR *Pseudomonas aerogonisa* infections [8] [9] [10]. On the other hand resistance to Colistin has also been reported by many countries in clinical isolates of *P. aeruginosa* [11] [12]. However in situation of Colistin resistance, it requires some other alternative solution such as combined therapy to fight with the multidrug resistance strains of *Pseudomonas aeruginosa.* Combination therapies usually are effective in situation of antibiotic resistance. Studies have shown the synergistic action of Colistin with other drugs on MDR *Pseudomonas aeroginosa* [13] [14]. Therefore the present study is an attempt to deduce synergistic activity of Streptomycin and Colistin on Colistin resistant *Pseu-*

domonas aeroginosa under *in vitro* conditions and to determine the incidence of multi drug-resistant *Pseudomonas aeroginosa* in Jazan region of Saudi Arabia.

2. Materials & Methods

Incidence of MDR *P. aeroginosa*: To discover the incidence of multidrug resistance peudomonal infections in Jazan region. A total of 174 case reports on *Pseudomonas aeroginosa* were collected from microbiology lab of hospitals in Jazan city and their antibiotic sensitivity for four anti Pseudomonal drugs such as Pipercillin, cefepime, ceftazidime and gentamycin were studied.

Collection of *Pseudomonas aeroginosa* cultures: Twenty nine clinical isolates of MDR *Pseuedomonas aeroginosa* along with their Identification & susceptibility reports were collected during year 2017-2018 from microbiology labs of hospitals in Jazan region, K.SA.

Identification of *P. aeroginosa* (MDR) isolates in Microbiology lab (College of applied medical sciences): All the 29 clinical isolates, multi drug resistant (MDR) were re-identified by culturing on cetrimide agar to study pigmentation and colony morphology. Gram staining of the colonies was performed to study the morphology of isolates. Biochemical identification was done by performing oxidase test [15].

Determination of Colistin resistance: Colistin sensitivity test was performed by disc diffusion technique. The isolates and reference strain of *Pseudomonas aeroginosa* (control) was sub cultured on Muller hinton agar plates and from each 24 hrs subculture plates an inoculum of 0.5 Mcfarland unit turbid suspension was prepared respectively. A sterile swab was dipped into each suspension and was spread on sterile Muller hinton agar plates. After spreading the inoculum 10 µg Colistin disc was placed in the center of the plates. The plates were then incubated at 37˚C for 24 hrs. After incubation the plates were observed for zone of inhibition. The zone of inhibition was measured in millimeter (mm). The susceptibility or resistance interpretations were determined in accordance to National Committee for Clinical Laboratory Standards (NCCLS) standard breakpoints [16].

Determination of MIC (minimum inhibitory concentration): All the 29 resistant cultures were tested by minimum inhibitory concentration technique. The protocol for MIC is preparation of two fold dilution of each drug (streptomycin alone and Colistin alone) and combination of two drugs (streptomycin-Colistin combined) which were prepared in Muller hinton broth tubes (macrobroth dilution technique). The concentrations ranging from 500 $\mu g \cdot ml^{-1}$ - 15.6 $\mu g \cdot ml^{-1}$ respectively was obtained [17].

Inoculum preparation for MIC: An inoculum adjusted to 0.5 McFarland turbidity was diluted to obtain a concentration of 5×10^5 CFU/mL *i.e.* 2.0 ml of the 0.5 McFarland suspension is diluted into 38 ml sterile water (1:20 dilution). Then 0.01 ml of the diluted suspension is delivered to each tube with Muller hinton broth with concentrations of antibiotics ranging from 500 $\mu g \cdot ml^{-1}$ - 15.6 $\mu g \cdot ml^{-1}$ respectively. After inoculation the tubes were incubated at 37˚C for 18

hrs. Inoculums for reference strains from American type culture collection (ATCC) were also prepared and tested for MIC as positive controls while tubes, without any inoculum was considered as negative control. After incubation the tubes were observed for visual turbidity. The lowest concentration of antibiotic or combined that completely inhibits visual growth of bacteria (no turbidity) was recorded as MIC [16].

The synergistic effect of streptomycin and Colistin: The synergy of Colistin and streptomycin at different concentrations on Colistin resistant *Pseudomonas aeroginosa* was determined by *FICI* (fractional inhibitory concentration index). *FICI* is described as the sum of the MIC of each drug when used in combination divided by the MIC of the drug used alone.

The formula to calculate *FICI* is as follows:

$$FICI = \left[\frac{\text{MIC drug A in combo}}{\text{MIC drug A alone}}\right] + \left[\frac{\text{MIC drug B in combo}}{\text{MIC drug B alone}}\right]$$

The Synergistic effect will be recorded when *FIC* indexes is ≤ 0.5, partial Synergy, if value < 1.0; additive when *FIC* = 1.0; indifferent when *FIC* > 1.0 but < 4.0 and antagonistic when *FIC* ≥ 4.0 [18] [19].

3. Results

Incidence of MDR *P. aeruginosa* (2017-2018):

Upon analyzing the data collected, it was determined that out 173 clinical cases reported 96 isolates *i.e.* (55.5%) were multidrug-resistant *P. aeroginosa* strains and 77 (44.5%), isolates were found to be sensitive to routinely used anti Pseudomonal drugs as shown in **Table 1**, **Figure 1**.

Colistin Resistance: The Zone of inhibition diameters obtained following the Colistin susceptibility test it was found that all the 29 isolates tested were resistant to Colistin antibiotic. According to NCCLS breakpoints a *Pseudomonas aeruginosa* strain is sensitive to Colistin if the zone diameter is ≥10 mm. In our study on 29 isolates, the zone diameters obtained were <10 mm as shown in **Table 2** & **Figure 2**

Synergistic activitity: Based on the *FICI* results obtained we found that the combination of Colistin and streptomycin showed synergistic killing of nineteen strains (55.1%) seven strains (20.6%) had partial synergistic action by the drugs. Similarly indifferent synergy was observed on five strains. Two strains showed additive synergy (6.8%) while two strains (3.4%) showed antagonism to the combined drugs as shown in **Table 3**.

4. Discussion

Treating multi drug resistance is the current major challenges for clinicians. It is reported that *Pseudomonas aeruginosa* is the most common non fermenting isolates in clinical samples in Saudi Arabia [20]. In the present study the incidence of *Pseudomonas aeruginosa* isolates causing infections were studied; it was found that 55.5% of the isolates were multi drug resistant; Among which

Table 1. Sensitivity pattern of *P. aeroginosa* against four anti pseudomonal drugs such as Pipercillin, cefepime, Ceftazidime, Gentamicin.

S. No.	Sample No.	Sample type	Antibiotic suceptibility											
			Piperacillin			Cefepime			Ceftazidime			Gentamicin		
			S	I	R	S	I	R	S	I	R	S	I	R
1	1	Wound		R			R			R			R	
2	2	Wound		S			I			I			R	
3	3	Sputum		R			R			R			I	
4	4	Sputum		R			I			R			R	
5	5	Wound swab		R			R			R			I	
6	6	Blood		I			S			S			S	
7	7	Sputum		I			S			S			S	
8	8	Throat swab		R			R			R			S	
9	9	Throat		R			R			R			S	
10	10	Wound swab		R			R			R			S	
11	11	Wound swab		R			I			I			S	
12	12	Urine culture		S			S			S			S	
13	13	Blood culture		I			S			S			S	
14	14	Blood		R			R			S			R	
15	15	Sputum		S			S			S			S	
16	16	Wound		S			S			S			S	
17	17	Blood		R			S			S			S	
18	18	Eye		R			R			R			R	
19	19	Sputum		I			S			S			S	
20	20	Wound		R			R			R			R	
21	21	Wound		I			R			R			R	
22	22	Wound		I			S			S			S	
23	23	Wound		S			S			I			S	
24	24	Throat		I			I			I			S	
25	25	Blood		R			S			I			S	
26	26	Urine		R			I			I			S	
27	27	Wound		I			S			S			S	
28	28	Wound		I			S			S			S	
29	29	Sputum		R			I			R			S	
30	30	Pus		I			S			S			S	
31	31	Blood		I			S			S			S	
32	32	Wound swab		I			S			S			S	
33	33	Wound Swab		I			S			S			S	

Continued

34	34	Sputum culture	I	S	S	S
35	35	Wound swab	R	R	R	R
36	36	Blood	R	R	R	R
37	37	Urine culture	I	S	S	S
38	38	Sputum	I	S	S	S
39	39	Sputum	I	I	I	S
40	40	Sputum	I	S	S	S
41	41	Wound swab	R	I	R	S
42	42	Wound swab	I	S	S	S
43	43	Wound swab	I	S	S	S
44	44	Wound swab	R	I	R	S
45	45	Urine culture	R	R	R	R
46	46	Sputum	R	R	R	S
47	47	Sputum	I	S	S	S
48	48	Wound swab	S	S	S	S
49	49	Blood culture	R	S	S	S
50	50	Wound swab	R	S	S	R
51	51	Blood culture	R	R	R	S
52	52	Urine culture	I	S	S	S
53	53	Urine culture	S	S	S	S
54	54	Contact lense	R	I	I	S
55	55	Wound swab	I	S	S	S
56	56	Urine culture	I	S	S	S
57	57	Sputum	R	I	R	S
58	58	Wound	S	S	S	S
59	59	Sputum	I	S	S	S
60	60	Urine culture	S	S	S	S
61	61	Sputum	I	S	S	S
62	62	Sputum	I	S	S	S
63	63	Sputum	S	S	S	S
64	64	Tip culture	S	S	S	S
65	65	Pus culture	R	S	R	I
66	66	Wound	R	R	R	R
67	67	Wound	R	S	S	R
68	68	Blood	R	R	R	S
69	69	Sputum culture	R	R	R	S
70	70	Blood	R	R	R	I

Continued

71	71	Wound	R	R	R	R
72	72	Throat swab	R	R	R	S
73	73	Wound	R	R	R	S
74	74	Throat swab	S	S	S	S
75	75	Sputum	R	R	R	R
76	76	Urine	I	S	S	S
77	77	Sputum	R	R	R	R
78	78	Wound	R	R	R	I
79	79	Sputum	R	R	R	R
80	80	Sputum	R	R	R	R
81	81	Blood	S	S	S	S
82	82	Urine	S	S	S	I
83	83	Urine	R	S	S	R
84	84	Urine	R	S	R	R
85	85	Blood	I	S	S	S
86	86	Urine	R	R	R	R
87	87	Throat	R	R	R	I
88	88	Wound	R	R	R	I
89	89	Sputum	R	R	R	R
90	90	Sputum	R	S	R	S
91	91	Urine	R	R	R	R
92	92	Urine	R	R	R	R
93	93	Sputum	R	R	S	R
94	94	Sputum	R	R	R	S
95	95	Eye swab	S	R	S	R
96	96	Sputum	R	R	R	R
97	97	Wound	R	R	R	R
98	98	Sputum	S	R	I	S
99	99	Urine	R	R	S	R
100	100	Sputum	S	R	R	R
101	101	Sputum	R	R	R	S
102	102	Sputum	R	R	R	S
103	103	Urine	I	S	S	S
104	104	Wound	R	R	R	S
105	105	Sputum	I	I	I	R
106	106	Eye	I	R	R	S
107	107	Wound	R	R	R	R

Continued

108	108	Sputum	S	S	S	S
109	109	Sputum	R	R	I	S
110	110	Sputum	R	R	R	R
111	111	Wound	I	I	R	S
112	112	Sputum	R	R	R	R
113	113	Sputum	I	S	S	S
114	114	Wound	R	I	I	S
115	115	Eye	S	R	R	S
116	116	Urine	R	I	S	I
117	117	Corneal pus	S	S	R	S
118	118	Stool	I	S	S	S
119	119	Wound	R	R	R	R
120	120	Sputum	R	R	R	S
121	121	Sputum	R	R	R	S
122	122	Sputum	R	R	R	S
123	123	Wound	I	S	S	S
124	124	Sputum	I	S	S	S
125	125	Sputum	S	S	S	S
126	126	Urine	R	I	R	S
127	127	Sputum	I	I	I	S
128	128	Conjuctive	I	S	S	S
129	129	Pus	I	I	I	S
130	130	Urine	S	S	S	S
131	131	Throat	S	S	S	S
132	132	Throat	R	R	R	S
133	133	Wound	I	I	I	S
134	134	Wound	R	R	R	R
135	135	Sputum	R	R	R	S
136	136	Pus	R	R	R	S
137	137	Wound	I	S	S	S
138	138	Wound	I	S	S	S
139	139	Wound	R	R	R	R
140	140	Sputum	S	S	S	S
141	141	Eye	R	R	R	S
142	142	Throat	I	I	I	R
143	143	Sputum	R	R	R	R
144	144	Sputum	S	R	I	S

Continued

145	145	Wound	R	I	R	R
146	146	Wound	S	S	S	S
147	147	Sputum	R	I	I	R
148	148	Throat	S	S	I	S
149	149	Throat	I	I	R	S
150	150	Wound	R	R	R	S
151	151	Pus	R	R	R	S
152	152	Urine	R	R	R	R
153	153	Urine	R	R	R	S
154	154	Wound	I	S	S	S
155	155	Sputum	R	R	I	R
156	156	Sputum	I	I	I	S
157	157	Sputum	R	R	I	R
158	158	Sputum	R	R	R	R
159	159	Wound	I	S	S	S
160	160	Wound	R	I	R	R
161	161	Wound	R	R	R	R
162	162	Pus	R	R	R	S
163	163	Throat	R	R	R	R
164	164	Wound	R	R	R	S
165	165	Sputum	R	R	R	R
166	166	Blood	R	R	R	S
167	167	Stool	I	I	R	S
168	168	Blood	I	I	I	R
169	169	Urine	S	I	R	R
170	170	Sputum	S	S	S	S
171	171	Sputum	R	R	R	R
172	172	Sputum	R	R	R	R
173	173	Wound	R	R	R	R

Legends: R—Resistant, SI—Sensitive, I—Intermediate.

22.5% of the isolated were resistant to all the anti Pseudomonal antibiotics (Pipercillin, Cefepime, Ceftazidime & Gentamycin) while 17.3% isolates were resistant to three commonly used anti Pseudomonal drugs such as Pipercillin, cefepime, Ceftazidime. In addition it was also analyzed that the highest percentage of multi drug resistant *P. aeruginosa* strains where associated with wound (20.2%) and respiratory tract infections (24%). while 5.7% of urine and 4% of blood isolates showed multi drug resistance. Our findings differ with the findings conducted in Riyadh Saud Arabia in which it was documented that most of

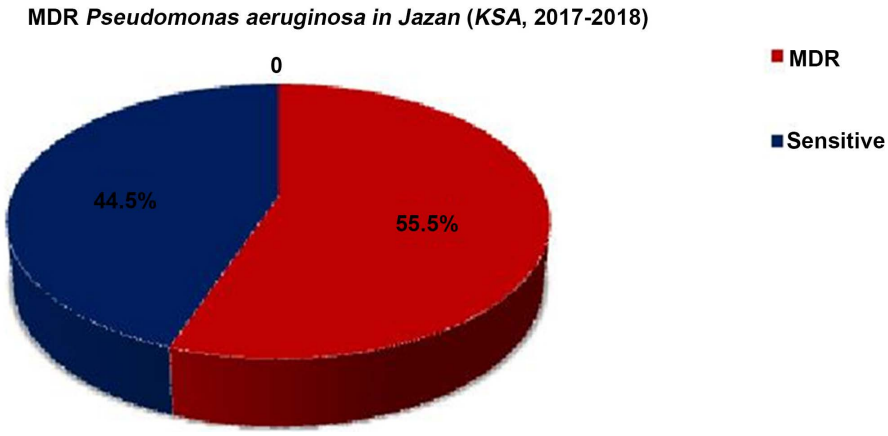

Figure 1. Incidence of MDR (multi drug resistant) *P. aeroginosa* in Jazan (2017-2018).

Figure 2. Plate of muller hinton agar (MHA) showcasing Colistin resistant *Pseudomonas aeruginosa.*

the MDR *P. aeruginosa* strains were associated with skin and wound swabs (47.3%) in 2004 & 2005; urine sample where the second most common samples to obtain MDR *P. aeruginosa* strains (26.3% & 33.3%) while respiratory specimens showed third common association with MDR *P. aeruginosa* (21% & 25%) in the respective studied years [21]. In our study the respiratory specimens (sputum and throat swabs) showed the highest percentage of association (24%) of MDR *P. aeruginosa* strains while wound swabs where the second most common specimens that showed association with MDR *P. aeruginosa* strains. In recent years Colistin is been used extensively to treat multidrug resistance *P. aeruginosa* infections. In the present study firstly we evaluated the effect of Colistin on twenty nine clinical isolates of multi drug resistance *Pseudomonas aeruginosa* collected from different hospitals of Jazan region of Saudi Arabia. To our surprise all the 29 clinical isolates showed Colistin resistance which is in contrary to results stated in study carried out in King Khalid University Hospital, Riyadh, Saudi Arabia (2012). In this study the researchers stated that 93.9% *P. aeruginosa* strains were susceptible to Colistin [22]. Colistin resistance is due to acquisition of

Table 2. Inhibition zones for Colistin susceptibility test (mm).

S. No.	Sample No.	Sample type	Antibiotic sensitivity of Colistin	
			Zone in mm	R/S
1	1	Wound	4	R
2	2	Wound	3	R
3	3	Sputum	5	R
4	4	Wound	4	R
5	5	Blood	5	R
6	6	Sputum	4	R
7	7	Throat swab	4	R
8	8	Throat swab	5	R
9	9	Wound	4	R
10	10	Wound	5	R
11	11	Urine	4	R
12	12	Blood	4	R
13	13	Blood	4	R
14	14	Sputum	2	R
15	15	Wound	4	R
16	16	Sputum	4	R
17	17	Eye	4	R
18	18	Sputum	5	R
19	19	Wound	4	R
20	20	Wound	5	R
21	21	Wound	4	R
22	22	Wound	5	R
23	23	Throat swab	3	R
24	24	Blood	5	R
25	25	Urine	4	R
26	26	Wound	4	R
27	27	Wound	4.5	R
28	28	Sputum	5	R
29	29	Sputum	5	R

mcr-1 gene in plasmid which was described by a study in china [23]. However *E. coli* strains harboring plasmids carrying mcr-1 gene have been reported from many parts of the world. For example in recent study in conducted in UAE found plasmids carrying mcr-1 gene in four strains of *E. coli* collected from Arabian peninsula [24]. Similarly in a study on hajj pilgrims demonstrated the presence of mcr-1 gene in 10 isolates of *E. coli* [25]. In another study on

Table 3. Fractional inhibitory concentration index (*FICI*) of twenty nine MDR *Pseudomonas aeruginosa* strains.

Sample number	MIC for Colistin	MIC for Streptomycin	MIC FOR CS	Colistin in combo	Strep in combo	*FICI* =
1	62.5	62.5	62.5	31.25	31.25	1
2	31.25	15.625	62.5	31.25	31.25	3
3	31.25	62.5	125	62.5	62.5	3
4	62.5	62.5	15.625	7.81	7.81	0.24
5	500	250	125	62.5	62.5	0.75
6	15.625	31.25	15.625	7.81	7.81	0.73
7	15.625	250	7.81	3.9	3.9	0.04
8	125	500	7.81	3.9	3.9	0.03
9	7.81	7.81	500	250	250	32.5
10	7.81	7.81	7.81	3.9	3.9	0.98
11	250	250	500	250	250	2
12	31.25	31.25	15.625	7.81	7.81	0.48
13	15.625	7.81	7.81	3.9	3.9	0.73
14	31.25	250	31.25	15.625	15.625	0.31
15	31.25	31.25	31.25	15.625	15.625	1
16	125	250	7.81	3.9	3.9	0.04
17	125	250	31.25	15.625	15.625	0.18
18	250	250	7.81	3.9	3.9	0.03
19	1000	31.25	7.81	3.9	3.9	0.12
20	15.625	7.81	7.81	3.9	3.9	0.73
21	15.625	500	7.81	3.9	3.9	0.5
22	31.25	31.25	7.81	3.9	3.9	0.48
23	15.625	15.625	7.81	3.9	3.9	0.48
24	15.625	15.625	7.81	3.9	3.9	0.48
25	15.625	31.25	7.81	3.9	3.9	0.37
26	125	250	62.5	31.25	31.25	0.37
27	62.5	62.5	31.25	15.625	15.625	0.5
28	15.625	7.81	7.81	3.9	3.9	0.74
29	62.5	7.81	31.25	15.625	15.625	2.25

Legends: MIC—minimum inhibitory concentration, CS—Colistin, *FICI*—fractional inhibitory concentration index.

emergence of antimicrobial resistance in Saudi Arabia stated that there may be more isolates of mcr-1 positive *E. coli* isolates in the country and it is most likely to have dissemination of mcr-1 conjugated plasmid to other species [26]. Therefore in our study we suggest that the Colistin resistance in the tested isolates may

be attributed to the acquisition of mcr-1 gene in *Pseudomonas aeruginosa* form mcr-1 positive *E. coli* strains. However a molecular investigation is required to prove it.

In the present study the synergistic action of two drugs *i.e.* Colistin and streptomycin was determined. It was found that the two drugs when combined showed synergistic killing of 55.1% of tested strains (*FICI* range 0.03 - 0.50). A number of studies have demonstrated the synergistic killing of *P. aeruginosa* strains by using different combinations of drugs for example in a study on synergistic activity of streptomycin and cefadroxil was observed to be synergistic against 39 strains of *P. aeruginosa* (*FICI* = 0.16 – 0.50) [19]. In another study Colistin when combined with antibiotics like trimethoprim, and other combined drugs like trimethoprim-sulfamethoxazole, or vancomycin showed synergistic killing of 12 tested *P. aeruginosa* isolates which included both multidrug resistant and Colistin resistant strains [27]. Similarly a study evaluated the synergistic activity of Colistin with dorepenem that showed substantial synergistic killing of Colistin-heteroresistant reference strain (ATCC 27853) and a Colistin-resistant MDR clinical isolate (19147 n/m) at a low inoculum of ~10^6 [13]. The studies pertaining to synergistic activity of Colistin with other drugs on Colistin resistant *P. aeruginosa* seems to suggest that Colistin resistance may encourage synergistic killing of Colistin resistant strains when combination includes Colistin and other antibiotics. However further research is required to understand the actual mechanism. Nevertheless *in vivo* trials also have showed that aerosolized Colistin is effective as supplemental therapy in patients with nosocomial pneumonia or trachea bronchitis resulting from MDR *P. aeruginosa* [8]. Hence Colistin may be included in the combination therapies to treat the infections caused by Colistin resistant MDR *P. aeruginosa*.

Our study is first of its kind to evaluate Colistin with streptomycin effect on MDR *Pseudomonas aeruginosa*. Streptomycin belongs to aminoglycosides and is used as anti tuberculous drugs. It inhibits protein synthesis in bacterial cell. However researchers have demonstrated the bactericidal activity of streptomycin against *Pseudomonas aeroginosa* [19] [28] but recent studies has demonstrated the developing streptomycin resistance *in Pseudomonas aeruginosa* [29] [30]. Therefore our study suggest that Colistin and streptomycin combined therapy may prove to be effective in treating *Pseudomonas aeruginosa* infections caused by MDR strains and as well as MDR strains that are resistant to Colistin and streptomycin antibiotics. Our study is also the first study report the antagonism between streptomycin and Colistin (*FICI* = 32.5). Therefore we recommend that it's very essential to choose the antibiotic combinations carefully and the combination should be evaluated before administration.

5. Conclusion and Recommendations

Based on data obtained we conclude that multi drug resistance Pseudomonal infections are on rise in this region (55.5%). In addition most of the MDR strains

(100% in our study) are Colistin resistant. Therefore care should be taken before reintroducing the use of Colistin to treat MDR Pseudomonal infections in this region. As well as antibiotic-resistant patterns should be considered before following the universal antibiotic guidelines. It is also recommended to use Colistin only after performing Colistin susceptibility test for the MDR isolates. Nevertheless an alternate solution of using combined therapy is also recommended to overcome the infections caused by this superbug. In addition use of Colistin and streptomycin combinations may provide a better therapeutic option to combat the MDR and Colistin resistant Pseudomonal infections but requires *in vivo* studies.

Authors' Contribution

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

Funding

None.

Data Availability

All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files.

Ethics Statement

This article does not contain any studies with human participants or animals performed by any of the authors.

Acknowledgements

The authors acknowledge Prince Mohammed bin Nasser hospital for its support during this work.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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