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In-Vitro Activities of Linezolid and Co-Trimoxazole against Isolates of Diverticulitis: Breakpoint Determinations

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ABSTRACT

Diverticulitis is a common colon disease with pouch like projection which leads to morbidity and mortality commonly cause by *E.coli and P. aeruginosa*.15 isolates of MDR *E.coli* and *P. aeruginosa* derived from patient of diverticulitis were identified using biochemical tests and EMB media. Their susceptibility, resistance, and MIC values were calculated using standard agar dilution method against Linezolid (linz) and co-trimoxazole (co-tri). Results revealed that at different concentrations (conc.) 0.5, 1, 2, 4, 8, 16mgl^{-1}, 1, 2, 4, 16, 32, and 64mgl^{-1} of linz and co-tri respectively. Linz and co-triMIC_{90} against *E.coli* was achieved ≥ 16mgl^{-1} and >64mgl^{-1}(P<0.01)respectively while against *P.aeruginosa*MIC_{90}wasachieved at 2.25mgl^{-1} and > 64mgl^{-1} respectively (P<0.01). At maximum conc. of 16mgl^{-1}and 64mgl^{-1} of linz and co-tri87.5 % and 75 % pathogens of *E.coli* were susceptible. *P .aeruginosa* at similar conc. gives 100 % and 71.42 % susceptibility (P<0.01).It is conclude that linz against current multidrug resistance (MDR) pathogens of diverticulitis was potent than co-tri.

Key Words: Diverticulitis, *Escherichia coli (E.coli),* and *Pseudomonas aeruginosa (P. aeruginosa),* Linz, Co-tri, and Clinical Isolates

INTRODUCTION

Diverticulitis is a common digestive disease of large intestine with formation of pouch like projection outside colon, diverticulum also known as a tubular sac or process branch off from a canal[1]. There are various conditions of diverticulitis i.e. complicated diverticulitis which is associated with fistula, obstruction, bleeding or perforations and this complicated condition is a main cause of morbidity and mortality[2]. From 15 to 20 % of cases of complicated diverticulitis are associated with abscesses[3], which could be manage with chemotherapy and percutaneous drainage[4],while uncomplicated diverticulitis could be mange by bowel rest and antimicrobial therapy. If patient could not respond to antimicrobial than a hospitalization will be suggested for IV antibiotic chemotherapy[5,6]. Generally diverticulum with obstruction becomes inflamed[1]. Pattern of occurrence of diverticulitis in current century has been increased[7]. It is one of the greatest common problem in western countries, and the prevalence quote that 60% cases are above age of 70 years[8].Intra-abdominal infections related with diverticulitis are related with aerobic and anaerobic pathogens *E.coli, Pseudomonas aeruginosa,* and *Klebsiella spp*[9].Among these three bacteria *Pseudomonas aeruginosa* is the highly resistant pathogen to antimicrobials. This pathogen even acquires such resistance as a result of chromosomal gene mutation. *Pseudomonas aeruginosa* also have the ability of combination of different mechanisms of resistances. Due to which recently *P. aeruginosa* come under definition of multidrug resistance (MDR) and extensively drug resistance (XDR) pathogen[10].Diverticulitis can be successfully treated without surgery. According to Sanford's antibacterial therapy 2003guideline co-tri is a good choice for

management of diverticulitis[1]. But frequent and over dose of these drugs made it more resistant. In current study linz and co-tri are used alone.

Objectives of the present study: To study the in vitro comparative activity of different concentrations of linz and co-tri against the multi-drug resistant pathogens of diverticulitis in population. Also to observe the effect and potential sensitivity pattern on the isolated pathogens of diverticulitis with increasing concentration of linz and co-tri.

MATERIAL AND METHOD

Pathogens, media and antibiotics: Total15 MDR isolates were taken out of which 8 were *E.coli (n*=8) *and*7 were *P. aeruginosa (n*=7).

Inclusion criteria: Patients between ages of 12 to 65 years were selected having diverticulitis of 3.5 moths.
In-vitro studies: These organisms were grown and subculture on EMB agar and were further identified by bio-chemical tests such as Indole, Methyl red, Voges-Proskauer, Citrate Utilization test and Catalase tests. MIC of linz and co-tri were determined against various isolates of diverticulitis using agar dilution method.

The sub grown cultures were then suspended in 1 ml Muller Hinton broth. After 2 hours the broth gets turbid shows bacterial growth and was matched with 0.5 MacFarland turbidity standards which contain 1×10^8 CFU/ml. Series of different concentrations i.e. 1, 2, 4, 16, 32, 64mgl^{-1} for co-tri and 0.5, 1, 2, 4, 8, and 16mgl^{-1} for linz were prepared in double distilled water, then these concentrations were mixed with liquid agar medium at (45 to 50^{o}C) in a ratio of 1:9 (one part of drug to 9 part of medium) and then this prepared solution was poured in to sterilized petri plates near flame and were allowed for solidification[11]. Petri dishes were arranged in order of increasing concentration of both drugs. Series of plates were prepared with addition of multiple inoculums by a applicators device[12]. After 24 hours' period of incubation, MIC and number of resistant strains were observed and results were calculated[13,14,15].

Statistical Analysis: Data was further analyzed using SPSS program according to one way ANOVA fallowed by test of homogeneity of variance. Values are considered significant when ($P<0.01$).

RESULTS

The patients of diverticulitis were between ages of 12-65 years.

MIC testing: Total 15 isolates multidrug resistance pathogens were collected which were based on individual antibiotics MIC evaluation. EMB media growth confirm gram negativity further bio-chemical tests i.e. Indole, Methyl red, Voges-Proskauer, Citrate Utilization test and Catalase tests confirm pathogens as a *E.coli* and *P. aeruginosa* as shown in table 1. The effect i.e. percent susceptibility of different concentrations 0.5, 1, 2, 4, 8, 16mgl^{-1}, 1, 2, 4, 16, 32, and 64mgl^{-1} of linz and co-trir respectively on *E.coli* and *P. aeruginosa* are shown in figure 3 and 4 respectively($P<0.01$).Linz was better than co-tri comparatively in both MDR pathogens. Homogeneity test of variance revealed that the data is highly significant. On an increasing concentration of linz comparatively with co-tri was significant. MIC_{50} and MIC_{90} of linz and co-tri are shown in table 2. Against both multidrug resistant pathogens i.e. *E.coli* and *P. aeruginosa* linz achieve at $MIC_{50\ and}$ MIC_{90} at $\geq$16mgl^{-1} and 2.25mgl^{-1} and co-tri achieve at $MIC_{50,}MIC_{90}$ at 4mgl^{-1},> 64mgl^{-1} respectively. With increasing concentrations of linz and co-tri resistance of pathogens of diverticulitis decreases which was more significant with linz in both pathogens as shown in figure 1 and 2.

DISCUSSION

Extensive work is being carried out in different areas of world on in-vitro activity of linz and co-tri against *E.coli* and *P. aeruginosa*. The purpose of current study was to identify the isolated pathogens of diverticulitis. For this purpose the isolated cutlers were identified as *E.coli* and *P. aeruginosa*. Their resistance, susceptibility MIC_{50} and MIC_{90} of these pathogens were calculated by agar dilution method. The isolated pathogens were gram negative because isolates were grown on EMB agar which only allows to grow Gram-ive pathogens. Further *P.aeruginosa* was identified by catalase test[16], while Indole, methyl red and Voges-Proskaur tests were negative for *P. aeruginosa* although all tests were positive for *E.coli*[17].

Previous studies suggests that *E.coli* was the prominent pathogen in diverticulitis[18], while *P.aeruginosa* is the most offending pathogen of diverticulitis for which co-tri is a drug of choice of the treatment of mild diverticulitis[19].But isolates of diverticulitis at different concentration shows maximum resistance even did not achieve MIC_{90} at 64mgl^{-1}. The isolates may be more resistant to co-tri.

Linz achieves MIC_{90} at 2.25mgl^{-1} against the P.*aeruginosa*. While in a wound infection linz achieve MIC at >100mgl^{-1}[20]. Linz against *E.coli* achieve MIC's at less than ≥ 16mgl^{-1}(P<0.01). In an in-vitro activity of linz with other drugs against *E.coli* achieves MIC at > 8microg/ml[21]. But linz achieve MIC_{50}at 1mgl^{-1} and MIC_{90} at ≥ 16mgl^{-1}. In a study of linz against *Fusobacterium spp* report that linz against *E.coli* at MIC>64mgl^{-1}is no active[22].Co-tri at concentration of 64mgl^{-1}could not achieve MIC_{90} against *P.aeruginosa* and resistance observed was about 28.57%. In a comparative study of co-tri against *P.aeruginosa* was about 77.3%[23]. This high concentration of co-tri is may be because of resistance development of pathogens of diverticulitis as patients of diverticulitis were already on broad spectrum antibiotics. Up to the author knowledge linz is not frequently used in diverticulitis in local population.

CONCLUSION

The pathogenesis of diverticulitis and resistance is a global health problem; particularly multi dug resistant pathogens are responsible for morbidity and mortality rate. These two pathogens are the most offending bacteria in diverticulitis. From the comparative study it is conclude that Linz may be a good choice in diverticulitis alone. Co-tri also gave satisfactory results.

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Table 1: Biochemical tests for identification of *E.coli and P. aeruginosa*

Specie	Indole Production test	Methyl red Test	Voges Proskaur test	Catalase Test
E.coli	+ive	+ive	-ive	+ive
P.aeruginosa	-ve	-ive	ive	+ive

Table 2: Cumulative susceptibility and MIC's of 15 multi-drug resistant strains of *E.coli and P. aeruginosa*

Microorganism	Antibiotics	Conc. Range	MIC_{50}	MIC_{90}	% Sensitivity
		mgl^{-1}	mgl^{-1}	mgl^{-1}	
E.coli (*n*=8)	Linz	0.5-16mgl^{-1}	1mgl^{-1}	≥16mgl^{-1}	87.5%
	Co-tri	1-64mgl^{-1}	2.25mgl^{-1}	>64mgl^{-1}	75%
P.aeruginosa(*n*=7)	Linz	0.5-16mgl^{-1}	0.875mgl^{-1}	2.25mgl^{-1}	100%
	Co-tri	1-64mgl^{-1}	4mgl^{-1}	>64mgl^{-1}	71.42%

Figure 1: Percent sensitivity of Linz against *E.coli* and *P.aeruginosa*

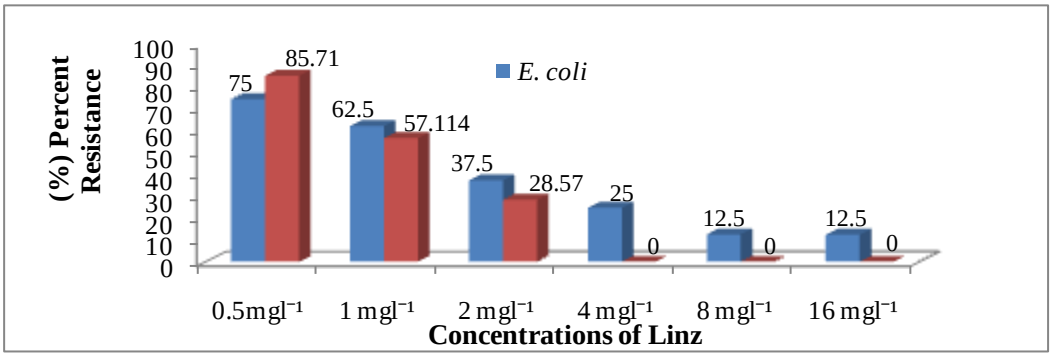


Figure 2: Percent sensitivity of Co-tri against *E.coli* and *P. aeruginosa*

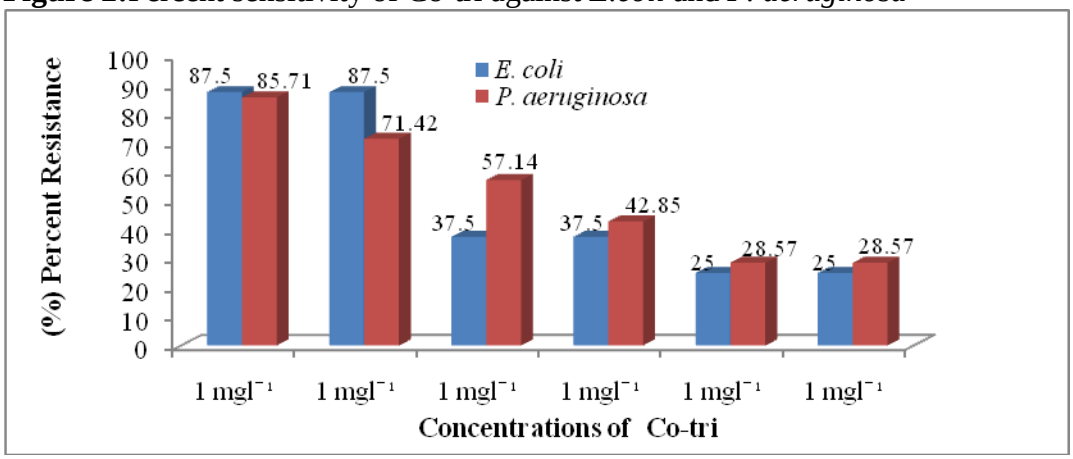


Figure 3:% sensitivity of Linz against *E.coli* and *P.aeruginosa*

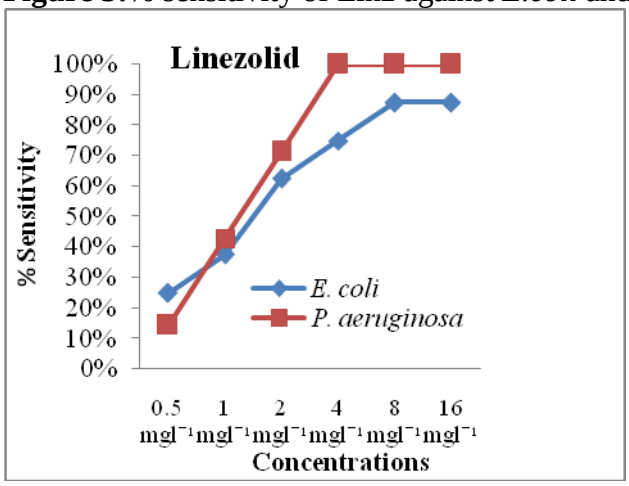
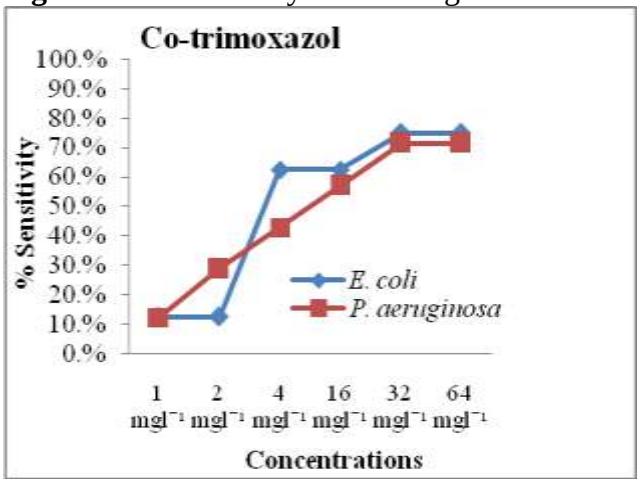


Figure 4: % sensitivity of Co-tri against *E.coli* and *P.aeruginosa*



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