In-Vitro Activities of Linezolid and Co-T 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-triMIC₉₀ against E.coli was achieved ≥ 16mgl⁻¹ and >64mgl⁻¹(P<0.01)respectively while against P.aeruginosaMIC₉₀was achieved at 2.25mgl⁻¹ and >64mgl⁻¹ respectively (P<0.01). At maximum conc. of 16mgl⁻¹and 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 pouch like projection outside colon, diverticulum also known as a tubular sac or process branch off from a canal¹. 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 mortality². From 15 to 20 % of cases of complicated diverticulitis are associated with abscesses³, which could be manage with chemotherapy and percutaneous drainage⁴,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 chemotherap⁵,⁶. Generally diverticulum with obstruction becomes inflamed⁷. Pattern of occurrence of diverticulitis in current century has been increased⁸. It is one of the greatest common problem in western countries, and the prevalence quote that 60% cases are above age of 70 years². Intra-abdominal infections related with diverticulitis are related with aerobic and anaerobic pathogens E.coli, Pseudomonas aeruginosa, and Klebsiella spp⁹. 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) pathogen¹⁰. Diverticulitis can be successfully treated without surgery. According to Sanford’s antibacterial therapy 2003 guideline co-tri is a good choice for
management of diverticulitis. 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:** Total 15 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 1×10⁸ CFU/ml. Series of different concentrations i.e. 1, 2, 4, 16, 32, 64 mg l⁻¹ for co-tri and 0.5, 1, 2, 4, 8, and 16 mg l⁻¹ for linz were prepared in double distilled water, then these concentrations were mixed with liquid agar medium at (45 to 50°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. 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. After 24 hours’ period of incubation, MIC and number of resistant strains were observed and results were calculated.

**Statistical Analysis:** Data was further analyzed using SPSS program according to one way ANOVA followed 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, 16 mg l⁻¹, 1, 2, 4, 16, 32, and 64 mg l⁻¹ of linz and co-tri 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. MIC₉₀ and MIC₉₀ 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 MIC₉₀ and MIC₉₀ at ≥16 mg l⁻¹ and 2.25 mg l⁻¹ and co-tri achieve at MIC₉₀/MIC₉₀ at 4 mg l⁻¹ ≥ 64 mg l⁻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 MIC₉₀ and MIC₉₀ 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, while Indole, methyl red and Voges-Proskaur tests were negative for *P. aeruginosa* although all tests were positive for *E.coli*.

Previous studies suggests that *E.coli* was the prominent pathogen in diverticulitis, 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. But isolates of diverticulitis at different concentration shows maximum resistance even did not achieve MIC₉₀ at 64 mg l⁻¹. The isolates may be more resistant to co-tri.
Linz achieves MIC90 at 2.25mg/l against the P.aeruginosa. While in a wound infection linz achieve MIC at >100mg/l. Linz against E.coli achieve MIC’s at less than ≥ 16mg/l (P<0.01). In an in-vitro activity of linz with other drugs against E.coli achieves MIC at > 8microg/ml. But linz achieve MIC at ≥ 16mg/l. In a study of linz against Fusobacterium spp report that linz against E.coli at MIC>64mg/l is no active. Co-tri at concentration of 64mg/l could not achieve MIC90 against P.aeruginosa and resistance observed was about 28.57%. In a comparative study of co-tri against P.aeruginosa was about 77.3%. 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 drug 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*

<table>
<thead>
<tr>
<th>Specie</th>
<th>Indole Production test</th>
<th>Methyl red Test</th>
<th>Voges Proskaur test</th>
<th>Catalase Test</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E.coli</em></td>
<td>+ive</td>
<td>+ive</td>
<td>-ive</td>
<td>+ive</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>-ve</td>
<td>-ive</td>
<td>ive</td>
<td>+ive</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Antibiotics</th>
<th>Conc. Range</th>
<th>MIC50</th>
<th>MIC90</th>
<th>% Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E.coli</em> (n=8)</td>
<td>Linz</td>
<td>0.5-16mg/l</td>
<td>1mg/l</td>
<td>≥16mg/l</td>
<td>87.5%</td>
</tr>
<tr>
<td></td>
<td>Co-tri</td>
<td>1-64mg/l</td>
<td>2.25mg/l</td>
<td>&gt;64mg/l</td>
<td>75%</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> (n=7)</td>
<td>Linz</td>
<td>0.5-16mg/l</td>
<td>0.875mg/l</td>
<td>2.25mg/l</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Co-tri</td>
<td>1-64mg/l</td>
<td>4mg/l</td>
<td>&gt;64mg/l</td>
<td>71.42%</td>
</tr>
</tbody>
</table>

**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*

![Graph showing percent sensitivity of Co-tri against E. coli and P. aeruginosa](image)

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

![Graph showing % sensitivity of Linz against E. coli and P. aeruginosa](image)

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

![Graph showing % sensitivity of Co-tri against E. coli and P. aeruginosa](image)
REFERENCES