Role of Azithromycin Against Clinical Isolates of Family Enterobacteriaceae: A Comparison Of Its Minimum Inhibitory Concentration By Three Different Methods

ABSTRACT

Purpose: To determine the effect of azithromycin, a new azalide antibiotic, on clinical isolates of the family Enterobacteriaceae and to determine and compare its minimum inhibitory concentration (MIC) by disk diffusion, agar dilution and E-test methods. Materials and Methods: One hundred fifty-nine bacterial strains belonging to the family Enterobacteriaceae, isolated from different clinical samples, were tested for their susceptibility to azithromycin by disk diffusion, agar dilution and E-test methods. The MIC values were analysed and the percentages of agreement between the different methods were mentioned. Results: Of the 100 isolates of the family Enterobacteriaceae, 47% were E. coli and Klebsiella species 47%, and Salmonella typhi 3% and 8%. Proteus mirabilis 2%, Proteus vulgaris 1%. Maximum isolates were obtained from urine 55/100 (55%). Azithromycin was found to be more active against Salmonella typhi, showing 100% sensitivity the by E-test and disk diffusion methods and agar dilution method. The overall agreement between disk diffusion and agar dilution method was 94%, between agar dilution and E-test was 86% and between disk diffusion and E-test was 89%. Conclusion: Azithromycin may become an important addition to our antimicrobial strategies, especially for the treatment of infections caused by Salmonella typhi and other infections caused by Enterobacteriaceae pathogens.

KEYWORDS

Azithromycin, enterobacteriaceae, minimum inhibitory concentration determination

Introduction

One of the major limitations to successful antimicrobial therapy of enteric bacterial pathogens has been the progressive emergence of resistance to these drugs, particularly in the developing countries. With a marked increase in antibiotic resistance among enteric bacterial pathogens, it has become imperative to find alternative effective antimicrobial agents. Among the oral antimicrobial agents, the fluoroquinolones and the broad-spectrum cephalosporins are the only groups whose efficacy against enteric pathogens of the family Enterobacteriaceae has not yet been compromised by acquired resistance. However, the fluoroquinolones are not yet recommended for use in pediatric patients because of articular damage caused by these drugs and the broad-spectrum cephalosporins because they are quite expensive and likely to induce TEM-like β lactamases, (TEM,named after patient Temoneira) which can hydrolyze the broad-spectrum cephalosporins.

Erythromycin is an old antibiotic that has been used to prevent infections caused by gram -ve enteric pathogens. Use of erythromycin is limited by frequent G.I side effects and because of high minimum inhibitory concentrations (MICs), which would presumably not be useful for treating infections caused by those members of the family Enterobacteriaceae that invade beyond the intestinal lumen (e.g., Salmonella and Shigella species). Azithromycin, a new azalide antibiotic, is active in vitro against a variety of gram -ve enteric bacterial pathogens. In murine typhoid models, azithromycin given once daily was highly effective in clearing the infection and this activity was attributable to the remarkable property of the intracellular concentration of azithromycin in the macrophage (>100 times than in serum). In the present study, different strains of the family Enterobacteriaceae were isolated from clinical samples. The antibiotic susceptibility pattern of different isolates with special reference to azithromycin was studied by the disc diffusion method. The MIC of azithromycin was evaluated by agar dilution and E-test methods.

Materials and Methods

The study was conducted in Department of Microbiology, S.V. Medical College, Tirupathi, on 100 isolated bacterial strains belonging to the family Enterobacteriaceae. All the clinical samples were inoculated into routine culture media and identification of the isolated organism was performed by standard procedures. Routine antimicrobial susceptibility testing of the bacterial strain belonging to the family Enterobacteriaceae was carried out by the Kirby-Bauer disk diffusion method. The MIC for azithromycin was determined by agar dilution and E-test methods. The vial containing azithromycin powder was obtained from Sigma aldrich, USA and dissolved in distilled water as stock solution according to the manufacturer’s instruction, which was used for the agar dilution method. The E-test strip for azithromycin was obtained from Hi-Media, India. The control organism Escherichia coli ATCC 25922 was included with each set of isolates tested, which was obtained from Department of Microbiology, SVMC, Tirupathi.

RESULTS

Among 100 isolates from the family Enterobacteriaceae, the bacterial strains isolated was 47(47%) E.coli, 47(47%) Klebsiella, 3(3%) Salmonella, 2(2%) Proteus mirabilis, 1(1%) Proteus vulgaris.

In the present study, different strains of the family Enterobacteriaceae were isolated from clinical samples. The antibiotic susceptibility pattern of different isolates with special reference to azithromycin was studied by the disc diffusion method. The MIC of azithromycin was evaluated by agar dilution and E-test methods.
(68.1%). The sensitivity of azithromycin to E.coli, Klebsiella and S.typhi is 31.9%,31.9% and 100% respectively. [Table 1]

On determination of the MIC of azithromycin by the agar dilution method among 47 strains of E.coli, 13 (27.7%) strains showed an MIC <8µg/ml and 25 strains showed an MIC≥25µg/ml. Among 47 strains of Klebsiella, 14(29.8%) strains showed an MIC <8µg/ml and 24 strains showed ≥32µg/ml. The Salmonella species showed 100% sensitivity. Proteus mirabilis and Proteus vulgaris showed 100% resistance, with MIC≥256µg/ml.

On determination of MIC of azithromycin by E-test method, 18 (38.3%) strains of E.coli showed an MIC <8µg/ml, 13(28%) strains showed MIC between 8-256 µg/ml and 16 strains (34.1%) showed MIC ≥256µg/ml. Among 47 strains of Klebsiella, 20(42.6%) showed MIC <8µg/ml, 22(47%) strains showed MIC between 8-256 µg/ml and 15 strains(32%) showed MIC ≥256µg/ml. Proteus mirabilis and Proteus vulgaris showed 100% resistance. Salmonella species showed 100% sensitivity to azithromycin.

By comparison of the MIC of Azithromycin, by agar dilution, E-test and disc diffusion method, E.coli were 31.9% sensitive by disc diffusion; 27.7% sensitive by agar dilution method and 38.3% by E-test. Klebsiella were 31.9% sensitive by disc diffusion, 42.6% by agar dilution and 100% by E-test. Proteus mirabilis and Proteus vulgaris showed 100% resistance. Proteus vulgaris showed 100% sensitivity to azithromycin.

On determination of MIC of azithromycin by E-test method, we found that imipenem was the most sensitive drug for all the isolates of the family Enterobacteriaceae, except Salmonella typhi, where azithromycin was found to be more sensitive. Although previous studies showed an excellent in vitro activity of azithromycin against the most common enteric bacterial pathogens, in our study, an increasing resistance of azithromycin to all the enteric bacterial pathogens except Salmonella typhi was observed. Thus, it was found to be the most effective antimicrobial agent for S. typhi.

DISCUSSION
Azithromycin penetrates into cells effectively and this intracellular penetration explains the therapeutic efficacy of this drug against the predominantly intracellular pathogen, like S. typhi. The ability of azithromycin to achieve intracellular concentration in monocytes is 231 times and in polymorphonuclear leucocytes is 83 times greater than that of serum concentration. It has a long half life of 2-3 days. The intracellular concentration appears to be essential for its therapeutic activity in typhoid fever. The availability of a paediatric suspension of azithromycin provides an opportunity to examine the efficacy and safety of this drug in young children with typhoid fever. In vitro resistance of azithromycin to different clinical isolates was reported to be 12% by Butler et al. In the present study, the antibiotic susceptibility pattern of 100 clinical isolates belonging to the family Enterobacteriaceae was studied. Among the 100 clinical isolates, 47 were E. coli (47%), and Klebsiella spp. 47 (47%). The majority of these isolates were obtained from urine samples. On studying the antibiotic susceptibility patterns of these clinical isolates by the disk diffusion method, it was found that imipenem was the most sensitive drug for all the isolates of the family Enterobacteriaceae, except Salmonella typhi, where azithromycin was found to be more sensitive. Although previous studies showed an excellent in vitro activity of azithromycin against the most common enteric bacterial pathogens, in our study, an increasing resistance of azithromycin to all the enteric bacterial pathogens except Salmonella typhi was observed. Thus, it was found to be the most effective antimicrobial agent for S. typhi.

The percentage of agreement between agar dilution and E-test is 86%, between disk diffusion and agar dilution is 94% and between disk diffusion and E-test is 89%. [Table 3]

Table 1: Antibiotic sensitivity pattern of different isolates of the family Enterobacteriaceae by disc diffusion method

<table>
<thead>
<tr>
<th>Organism</th>
<th>AK</th>
<th>AMC</th>
<th>CIP</th>
<th>CAZ</th>
<th>IPM</th>
<th>NA</th>
<th>AZM</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli</td>
<td>28 (59.6%)</td>
<td>0 (0%)</td>
<td>11 (23.4%)</td>
<td>1 (2.1%)</td>
<td>29 (61.7%)</td>
<td>7 (15.3%)</td>
<td>3 (6.4%)</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>23 (48.9%)</td>
<td>3 (6.4%)</td>
<td>17 (36.2%)</td>
<td>1 (2.1%)</td>
<td>24 (51.1%)</td>
<td>5 (10.6%)</td>
<td>4 (8.5%)</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>1 (100.0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>S.typhi</td>
<td>3 (100.0%)</td>
<td>0 (0%)</td>
<td>3 (100.0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>3 (100.0%)</td>
<td></td>
</tr>
</tbody>
</table>

AK – Amikacin, AMC–Amoxycillin/Claulanic acid, CIP–Ciproflloxacin, CAZ–Cefazidime, IPM–Imipenem, NA–Nalidixic acid, AZM–Azithromycin

Table 2: Comparison of susceptibility of azithromycin by various isolates by disc diffusion, agar dilution and E-test methods

<table>
<thead>
<tr>
<th>Organism</th>
<th>Agar dilution method</th>
<th>E-test method</th>
<th>Disk diffusion method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Susceptible</td>
<td>Resistant</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>14 (29.8%)</td>
<td>32 (70.2%)</td>
<td>20 (42.6%)</td>
</tr>
<tr>
<td>E.coli</td>
<td>34 (72.3%)</td>
<td>13 (27.7%)</td>
<td>18 (38.3%)</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>2 (100.0%)</td>
<td>0 (0%)</td>
<td>2 (100.0%)</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (100.0%)</td>
</tr>
<tr>
<td>S.typhi</td>
<td>3 (100.0%)</td>
<td>0 (0%)</td>
<td>3 (100.0%)</td>
</tr>
</tbody>
</table>

Table 3 : Percentage of agreement between the different methods (n=100)

<table>
<thead>
<tr>
<th>Test method</th>
<th>Strains showing sensitivity by both methods</th>
<th>Strains showing resistance by both methods</th>
<th>Percentage of agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD and AD</td>
<td>27</td>
<td>67</td>
<td>94.0</td>
</tr>
<tr>
<td>AD and E-test</td>
<td>27</td>
<td>59</td>
<td>86.0</td>
</tr>
<tr>
<td>DD and E-test</td>
<td>30</td>
<td>59</td>
<td>90.0</td>
</tr>
</tbody>
</table>

Percentage of agreement =A+B/total no.of isolates of Enterobacteriaceae in the study x 100; AD-agar dilution; DD-disc diffusion; E-epsilometer

REFERENCES
1. Murray BE. Resistance of Shigella, Salmonella, and other selected enteric path-
ogens to antimicrobial agents. Rev Infect.

2. Murray BE. New aspects of antimicrobial resistance and the resulting therapeu-

3. Andremont A., Raibaud P, Tancrede C. Effect of erythromycin on microbial an-
tagonsisms: A study in gnotobiotic mice associated with a human fecal flora. J

4. Gordillo ME, Singh KV, Murray BE. In vitro activity of azithromycin against bac-

5. Butler T, Girard AE. Comparative efficacies of azithromycin and Ciprofloxacin
against experimental Salmonella typhimurium infection in mice. J Antimicrob

6. Betty AF, Sahm D.F, Weissfeld AS, editors. Laboratory cultivation and isolation

7. Betty AF, Sahm D.F, Weissfeld AS, editors. Overview of conventional methods
for bacterial identification. Chapter 11. In: Bailey and Scott's diagnostic microbi-

8. Wildfeuer A, Loufen H, Zimmermann T. Uptake of azithromycin by various cells
and its intracellular activity under in-vivo conditions. Antimicrob Agents Chem-

9. Pascual A, Corejo MC, García I, Perea EI. Factors affecting the intracellular ac-

ment of typhoid fever with azithromycin versus chloramphenicol in a rand-

11. Gordillo ME, Singh KV, Murray BE. In vitro activity of azithromycin against bac-

parison of the E-test and agar dilution for in vitro antimicrobial susceptibility

al. Comparison of agar dilution, disk diffusion, microscan, and vitek antimicro-
bial susceptibility testing methods to broth microdilution for detection of fluoro-
quinoles: Resistant isolates of the family enterobacteriaceae. J Clin Microbiol

14. Mirza SH, Beeching NJ, Hart CA. Multi-drug resistant typhoid: A global prob-

1992;82:91-100.

16. N Chayani, S Tiwari, G Sarangi, B Mallick, A Mohapatra, BP Paty, P Das
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comparison of its Minimum inhibitory concentration by three different meth-
ods.