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

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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%,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. [1] 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 paediatric 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.[2]

Erythromycin is an old antibiotic that has been used to prevent infections caused by gram -ve enteric pathogens. [3] 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. [4] 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). [5]

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 Micro biology,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. [6],[7] 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.

On routine antimicrobial susceptibility testing by the disc diffusion method, E.coli and Klebsiella were more sensitive to Imipenem (61.7% and 51.1%) followed by amikacin (59.6% and 48.9%), respectively. Salmonella.typhi showed equal sensitivity to azithromycin, amikacin, amoxicillin/clavulonic acid, ciprofloxacin and imipenem i.e., 100%. Proteus.vulgaris was most sensitive to imipenem. In comparison with other isolates Proteus.spp. showed maximum resistance to azithromycin (100%), followed by E.coli (68.1%) and Klebsiella spp.

(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 diltion method among 47 strains of E.coli, 13 (27.7%) strains showed an MIC <8µg/ml and 25 strains showed an MIC≥32µg/ml. Among 47 strains of Klebsiella, 14(29.8%) strains showed an MIC <8µg/ml and 24 strains showed ≥32µg/m. 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, 29.8% by agar dilution and 42.6% by E-test. Salmonella were 100% sensitive by disc diffusion, 100% sensitive by agar dilution and 100% by E-test. Proteus.mirabilis and Proteus.vulgaris showed 100% resistance by all the three methods.[Table 2]

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

Organism	AK	AMC	CIP	CAZ	IPM	NA	AZM
E.coli	28 (59.6%)	0 (.0)	11 (23.4%)	1 (2.1%)	29 (61.7%)	7 (23.3%)	15 (31.9%)
Klebsiella	23 (48.9%)	3 (6.4)	17 (36.2%)	4 (8.5%)	24 (51.1%)	5 (20%)	15 (31.9%)
Proteus mirabilis	0 (.0)	0 (.0)	0 (.0)	0 (.0)	0 (.0)	-	0 (0)
Proteus vulgaris	1 (100.0%)	0 (.0)	0 (.0)	0 (.0)	1 (100.0%)	-	0 (0)
S.typhi	3 (100.0%)	3 (100.0)	3 (100.0%)	0 (.0)	3 (100.0%)	-	3 (100.0%)

AK –Amikacin, AMC-Amoxycillin/Clavulanic acid, CIP-Ciprofloxacin, CAZ-Ceftazidime,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

Organism	Agar dilution method		E-test method		Disk diffusion method	
	Susceptible	Resistant	Susceptible	Resistant	Susceptible	Resistant
Klebsiella	14 (29.8%)	33 (70.2%)	20 (42.6%)	27 (57.4%)	15 (31.9%)	32 (68.1%)
E.coli	13 (27.7%)	34 (72.3%)	18 (38.3%)	29 (61.7%)	15 (31.9%)	32 (68.1%)
Proteus. mirabilis	0(.0)	2 (100.0%)	0(.0)	2 (100.0%)	0(.0)	2 (100.0%)
Proteus. vulgaris	0(.0)	1 (100.0%)	0(.0)	1 (100.0%)	0(.0)	1 (100.0%)
S.typhi	3 (100.0%)	0(.0)	3 (100.0%)	0(0)	3 (100.0%)	0(.0)

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

Test method	Strains showing sensitivity by both methods	Strains showing resistance by both methods	Percentage of agreement
	A	B	
DD and AD	27	67	94.0
AD and E-test	27	59	86.0
DD and E-test	30	59	89.0

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

DISCUSSION

Azithromycin penetrates into cells effectively and this intracellular penetration explains the therapeutic efficacy of this drug against the predominantly intracellular pathogens 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. [8],[9] 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. The in vitro resistance of azithromycin to different clinical isolates was reported to be 12% by Butler etal . [10] 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 spps. 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, [11] in our study, an increasing resistance of azithromycin to all the enteric bacterial pathogens except Salmonella typhi was obtained. Thus, it was found to be the most effective antimicrobial agent for S.typhi

On comparison of the MIC by the different test methods, it was found that MICs obtained using the E-test was lower than those by agar dilution regardless of the species of organism tested. The correlation between the E-test and the agar dilution MICs varied greatly depending on the antimicrobial agent tested. [12] If the performance of these test methods is ranked by the percentage agreement of the interpretive results with the consensus interpretive results (highest agreement ranked best), the rank order would be E-test>disk diffusion>agar dilution. [13] Our study also showed a similar type of result. In our study, the percentage of agreement between agar dilution and E-test is 86%, between disk diffusion and agar dilution is 96.8% and between disk diffusion and E-test is 91.2%. The results in our study are nearly correlating to the study conducted by Chayani et al[16],. the overall agreement between disc diffusion and E-test was 91.2%, disc diffusion and agar dilution was 96.8%, agar dilution and E-test was 88%.[16] Emergence of antimicrobial drug resistance in S. typhi is a global problem [14],[15] and was the compelling reason to undertake the trial of a new alternative antibiotic for typhoid fever. This study showed an excellent invitro activityof azithromycin against Salmonella and Shigella species . Because of its good intracellular activity and relatively low MICs for enteropathogens, this drug may become an important addition to our antimicrobial strategies for the treatment of typhoid fever and also for bacillary dysentery. Further studies to define its role in the management of enteric bacterial infections may be required.

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