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Case Report

A case of clinical and microbiological failure of azithromycin therapy in Salmonella enterica serotype Typhi despite low azithromycin MIC

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ABSTRACT

Typhoid fever remains a serious problem in many developing countries. Due to resistance to multiple first line drugs, azithromycin has evolved as an important drug in the treatment of typhoid. While therapy with azithromycin is highly effective, no clinically validated mean inhibitory concentration (MIC) break points or disc diffusion cutoff guidelines are available so far. We describe an Indian adult with clinical and microbiological failure to azithromycin despite low azithromycin MIC.

1. Introduction

Typhoid fever caused by *Salmonella enterica* serotype Typhi remains an important cause of acute febrile illness in the developing countries, and Indian subcontinent remains the typhoid capital of the world. More than 20 million new cases of typhoid fever are reported to occur every year.[1] Due to extensive resistance to fluoroquinolones and other first line agents, azithromycin and third generation cephalosporins have evolved as first line treatment of uncomplicated enteric fever in many endemic countries. There are no clinically validated MIC break points or disc diffusion cutoff guidelines for interpreting azithromycin susceptibility in enteric fever either by CLSI or EUCAST. Epidemiological surveillance of bacterial susceptibility patterns have shown that isolates with MICs less than 16 microgram/ml are likely to be susceptible.[2] In view of the above limitations, documentation of patients with failure of azithromycin therapy is of paramount importance. Here, we report a young man from India with clinical failure of azithromycin therapy infected with *Salmonella enterica* serotype Typhi, which was susceptible to azithromycin *in-vitro* (MIC-4microgram/ml).

A twenty year old Indian male presented with high grade fever with chills and vomiting for three days' duration. His blood cultures sent on day three of fever grew gram negative bacilli that were subsequently identified as *Salmonella* Typhi (B7766). Awaiting susceptibility results, he was started on azithromycin 20 mg/kg intravenously daily on day five of illness. The organism was subsequently reported susceptible to Co- trimoxazole, Ampicillin, Chloramphenicol, Ceftriaxone and Azithromycin and resistant to Nalidixic acid and Ciprofloxacin. He continued to have fever and had worsening liver enzymes even after eight days of Azithromycin. Medication review revealed no potential drug interactions. Careful clinical examination revealed no evidence of metastatic abscesses or murmurs. His CT thorax, abdominal ultrasound and bone scan did not reveal any evidence of metastatic foci. The azithromycin MIC of *S. Typhi* was found to be 4 microgram/ml and was in the susceptible range. The MIC of the isolate was confirmed both by broth micro dilution testing and E test. The blood culture sent on day 10 of azithromycin treatment remained positive for *S. Typhi*, and he continued to have fever. The MIC of the organism was confirmed multiple times (including the day 10 blood culture sample) to be 4 microgram/ml. Quality controlled strains (*Staphylococcus aureus* ATCC 29213 – expected

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range 0.5 to 2 microgram/ml) were used in all batches for MIC detection to rule out faulty E test batches.

In view of clinical and microbiological failure, Azithromycin was stopped and he was started on a combination of Ceftriaxone, Cotrimoxazole and Chloramphenicol. Subsequently, his blood cultures became sterile on day 12 and he defervesced. His stool cultures after eight weeks remained sterile, and he did not have any evidence of relapse of typhoid fever.

To further study the mechanism behind the clinical failure, next generation sequencing (NGS) for *S. Typhi* (B7766) was performed. Genomic DNA was extracted and whole genome sequencing was performed using Ion Torrent (PGM) sequencer with 400-bp read chemistry (Life Technologies) according to manufacturer's instructions. Downstream analysis was performed in the CGE server (http://www.cbs.dtu.dk/services), RAST and PATRIC. This Whole Genome Shotgun project was deposited at DDBJ/ENA/GenBank under the accession LXWB00000000. The *S. Typhi* B7766 was identified to be sequence type (ST) 1 as analysed by MLST 1.8 tool (https://cge.cbs.dtu.dk//services/MLST/). The whole genome sequencing has been analyzed for presence of any antimicrobial resistance genes through ResFinder 2.1 (https://cge.cbs.dtu.dk//services/ResFinder/). The genome was negative for any common resistance genes except *mac*A and *mac*B. Though *mac*A and *mac*B gene corresponding to efflux pumps were found in the whole genome sequence test strain B7766, phenotypic assay with efflux pump inhibitor (PaβN) did not confirm the presence of efflux activity.

The efficacy data for Azithromycin in uncomplicated typhoid fever is from multiple randomized controlled trials. Cefixime and Ceftriaxone are also commonly used, however they have slower fever clearance times (FCTs) and a slightly higher relapse rate compared to Azithromycin.[3] It is in this context that our report of therapeutic failure with Azithromycin with MICs in the susceptible range is of significance.

Azithromycin treatment failure has been documented in *Salmonella Paratyphi A* in a few case reports; clinical and microbiological failure in *S. enterica* serovar Typhi is not yet reported. Though macrolide efflux pumps *mac* A and *mac* B were found in our isolate, phenotypic testing in the presence of an inhibitor did not confirm efflux pump activity. In multidrug resistant Neisseria gonorrhea expressing *mac* A and *mac* B, no demonstrable fall in the MICs after inactivation of the *mac* A and *mac* B efflux pumps has been recorded.[4] Even though this offers a potential explanation for the treatment failure recorded in our patient, further studies are required to confirm this observation. Azithromycin reaches 50 to 100 fold concentration within the macrophages and polymorphonuclear (PMN) cells (crucial in treating the predominantly intracellular organisms of enteric fever) but achieves very low plasma levels. Hence, MIC might not be a good indicator of decreased susceptibility to Azithromycin. In retrospect, it is highly likely that the patient could have been treated effectively by Ceftriaxone monotherapy itself. However, in view of persistent fever and documented microbiological failure even on day 10 of therapy, a clinical decision to use combination therapy was made.

Salmonella isolates with elevated Azithromycin MICs have been repeatedly documented among returning travelers from India[5] and also in studies from the Indian population.[6] However, the clinical relevance of these elevated MICs remains unknown. Clinically and microbiologically correlated breakpoints by Parry et al suggest that Azithromycin MIC of less than 16 microgram/ml likely predicts a favourable clinical outcome.[7]

To the best of our knowledge, this is the first report of treatment failure with Azithromycin, when the MIC of Azithromycin for *S. enterica* serovar *Typhi* was within the susceptible range. At present, Azithromycin has evolved as a preferred agent for treatment of typhoid fever with multidrug resistant isolates. In this scenario, careful monitoring and documentation of such clinical failures is of great importance.

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