Isolation of multidrug-resistant *Salmonella* in Singapore

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ABSTRACT Multidrug-resistant *Salmonella* is a well-recognised problem worldwide, especially in developing countries such as India, where non-typhoidal *Salmonella* infections and enteric fever are endemic. Antimicrobial resistance, particularly to fluoroquinolones, is common and leads to the frequent use of alternative agents, such as azithromycin. We herein describe the first reported case of azithromycin-resistant *Salmonella* gastroenteritis in a Singaporean patient.

Keywords: azithromycin, enteric fever, resistance, salmonella

INTRODUCTION

Salmonella species are one of the major causes of food-borne illnesses worldwide and pose an important public health problem. On a global scale, *Salmonella* accounts for approximately three billion human infections each year. The World Health Organization (WHO) has estimated that typhoid fever contributes to 21.7 million illnesses per year (217,000 deaths), with paratyphoid fever accounting for 5.4 million of these cases.[1]

Many serotypes of the *Salmonella* species have been identified. They can be broadly divided into two groups: typhoidal and non-typhoidal. The typhoidal group (e.g. *Salmonella enterica* serovars Typhi and Paratyphi A and B) causes enteric fever, while the non-typhoidal group (e.g. *Salmonella enterica* serovar Enteritidis) causes largely uncomplicated enterocolitis.

Although antimicrobial treatments are not indicated for self-limiting uncomplicated *Salmonella* infections in patients without underlying comorbidities and non-immunocompromised patients, they are necessary for invasive *Salmonella* infections.[1,2] Without treatment, enteric fever carries a mortality rate of 30%, while appropriate antimicrobial treatment reduces the mortality rate to as low as 0.5%.[1] At present, due to the widespread emergence of strains resistant to traditional first-line drugs such as ampicillin, chloramphenicol and trimethoprim, current recommendations suggest using fluoroquinolones (i.e. ciprofloxacin) or extended-spectrum cephalosporins (i.e. ceftriaxone) for the treatment of invasive and severe *Salmonella* infections.[1,2] However, the extensive use of fluoroquinolones has been associated with decreased susceptibility and resistance to this class of drugs. Several clinical trials have demonstrated the superior efficacy of azithromycin compared with fluoroquinolones and extended-spectrum cephalosporins.[3,4] We report the first case of azithromycin-resistant *Salmonella* isolated in Singapore.

CASE REPORT

A 65-year-old Chinese man presented to the accident and emergency department with a one-week history of fever, non-productive cough and exertional dyspnoea with decreased effort tolerance. His past medical history included hypertension, old stroke disease and Parkinson's disease with mild functional limitation. He had no prior travel history in the preceding three months.

The patient was initially treated for bilateral community-acquired pneumonia but subsequently tested positive for human immunodeficiency virus (HIV) infection. His baseline CD4 count was 15 cells/mm^3 and HIV-1 RNA load was 368,501 copies/mL (5.57 $\log_{10}$). Diagnostic bronchoscopy with bronchoalveolar lavage (BAL) was performed and confirmed the diagnosis of *Pneumocystis jirovecii* pneumonia. He was treated with three weeks of trimethoprim/sulfamethoxazole (TMP/SMX) and started on a highly active antiretroviral therapy regimen consisting of tenofovir, emtricitabine and efavirenz. In view of the patient's significant functional decline, he was transferred to a community hospital for rehabilitation one week later. However, he was transferred back to the acute hospital after two days due to an episode of fever and desaturation, which were attributed to aspiration pneumonia. He was started empirically on piperacillin-tazobactam. Antibiotic therapy was subsequently escalated to meropenem. Intravenous ganciclovir was started presumptively for the treatment of possible cytomegalovirus (CMV) pneumonitis in view of the positive CMV results for both polymerase chain reaction (PCR) (in the previous BAL) and antigenemia tests (CMV cytospin [pp65 antigen] was 1,462 in one million cells). The patient was closely monitored in the high-dependency unit for two days before being transferred to the general ward. No ventilatory support was required.

Since the transfer from the community hospital, the patient had been having loose non-bloody stools with no complaints of abdominal pain, nausea or vomiting. The initial stool culture and *Clostridium difficile* (CD) toxin test were negative. The patient received meropenem for four days; when his fever resolved and blood cultures yielded no significant bacterial growth, the antibiotic therapy was de-escalated to cefepime. His diarrhoea, however, persisted. Stool cultures repeated four days later grew *Salmonella enterica* serovar Stanley, which was susceptible to ampicillin, ceftriaxone and carbapenems, with intermediate susceptibility to ciprofloxacin and resistance to TMP/SMX. PCR testing for CD was negative. The stool specimen was also negative for ova, cysts, parasites, *cryptosporidium*, *cyclospora* and

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cystoisospora. To treat the *Salmonella* enteritis, antibiotic therapy was de-escalated to ceftriaxone, but the patient's diarrhoea persisted despite receiving five days of ceftriaxone. CMV cytospin, repeated after a week of ganciclovir, was 24 in one million cells.

Computed tomography of the abdomen and pelvis showed no significant mural thickening of the colon that was suggestive of colitis. Stool culture, repeated after eight days of antibiotic therapy, again isolated *Salmonella* Stanley, which showed resistance to ampicillin, TMP/SMX, ciprofloxacin (minimum inhibitory concentration [MIC] 2 mcg/mL), ceftriaxone (MIC > 256 mcg/mL) and azithromycin (MIC 32 mcg/mL). The extended-spectrum beta-lactamase (ESBL) confirmatory test was positive, with a CTX-M phenotype. The isolate was susceptible to only carbapenems. Upon retesting, the first *Salmonella* Stanley isolate was also found to be resistant to azithromycin (MIC 64 mcg/mL). Susceptibility testing was performed using the Clinical and Laboratory Standards Institute (CLSI) disk diffusion method. MIC was determined using Etest (bioMérieux, Marcy l'Etoile, France) and also interpreted according to the CLSI document (except for azithromycin, for which there are no CLSI criteria).[5] Repeated CD PCR was negative. Additional blood cultures showed no bacterial growth. The patient was treated with three days of ertapenem, after which his diarrhoea resolved. He was discharged after physiotherapy and caregiver training. The patient was also advised about adherence to antiretroviral therapy.

DISCUSSION

We report the first clinical case of azithromycin-resistant *Salmonella* isolated in Singapore. The isolate also showed resistance to ciprofloxacin and ceftriaxone. We postulate that strains of *Salmonella* with increasing resistance were isolated in subsequent stool cultures either due to infection with a mixed population of *Salmonella* or a *de novo* mutation as a result of selective pressure exerted by broad-spectrum antibiotics.

Prior to this case, there have been sporadic reports of strains of *Salmonella* Paratyphi A with azithromycin resistance in other parts of the world, especially in developing countries such as India and Cambodia, where salmonellosis is endemic.[6-9] Ciprofloxacin has been the first-line antibiotic of choice for the treatment of *Salmonella* infections since the widespread emergence of multidrug-resistant *Salmonella* Typhi; in the 1980s, the bacterium was found to be resistant to chloramphenicol, ampicillin and cotrimoxazole.[1,2] However, from the 1990s, there has been a rising incidence of treatment failures, with decreased ciprofloxacin susceptibility (DCS) and fluoroquinolone resistance in *Salmonella* isolates, possibly due to pervasive and inappropriate antibiotic prescription practices.[1] The mechanism of fluoroquinolone resistance occurs via a mutation in DNA gyrase. Indeed, this is a cause for concern, especially in endemic areas where ciprofloxacin remains the mainstay of treatment.[1] The emergence of DCS and fluoroquinolone resistance has prompted the increased use of third-generation cephalosporins in the treatment of invasive *Salmonella* infections. Recently, there have been sporadic reports of a high level of resistance to ceftriaxone, as CTX-M-15 ESBLs were found in *Salmonella*.[10]

Azithromycin is an azalide antimicrobial agent that has been demonstrated in clinical trials to be equivalent or superior to chloramphenicol, fluoroquinolones and extended-spectrum cephalosporins for the management of uncomplicated typhoid fever.[3,4] *In vitro* studies have demonstrated azithromycin's excellent tissue penetration and ability to achieve high intracellular concentrations (> 100 times higher than serum) in macrophages and neutrophils, coupled with a long half-life of 2–3 days.[11,12] Thus, it has been increasingly favoured for the management of bacillary dysentery and invasive non-typhoidal *Salmonella* infections. However, the clinical breakpoints for *Salmonella* and azithromycin have yet to be defined. The European Committee on Antimicrobial Susceptibility Testing states that isolates with an MIC < 16 µg/mL for azithromycin should be considered as wild-type organisms that are responsive to treatment.[13] In previous studies of typhoidal *Salmonella* isolates, MICs for azithromycin ranged from 4–64 µg/mL.[14]

Macrolide resistance has often been reported due to the expression of macrolide 2'-phosphotransferase encoded by the *mph*(A) gene.[10] It has been hypothesised that *Escherichia coli* may constitute a major reservoir from which plasmid-mediated transfer of resistance genes occur.[14] In the present case, molecular analysis for *mph*(A) gene in the *Salmonella* Stanley isolate was negative. Nonetheless, other genes known to be associated with macrolide resistance have yet to be looked into: *erm* genes are responsible for methylases causing target site modification; *ere*(A) and *ere*(B) genes encode for esterases for macrolide inactivation; and *mef*(A) and *msr*(A) genes cause acquisition of efflux pumps.[15]

Azithromycin is a useful alternative to ciprofloxacin for outpatient treatment of uncomplicated salmonellosis whose strains have demonstrated reduced susceptibility to fluoroquinolones. The isolation of azithromycin-resistant *Salmonella* in Singapore is a worrying development, especially in the absence of recent travel in the present case, as it suggests a locally acquired isolate. Recurrent salmonellosis is an AIDS-defining illness. The emergence of multidrug-resistant *Salmonella* is possibly the result of an increased use of antibiotics, especially macrolides, TMP/SMX and cephalosporins, both for the treatment of common infections and as a prophylaxis for HIV-positive patients with low CD4 counts (i.e. azithromycin for *Mycobacterium avium* complex and TMP/SMX for *Pneumocystis jirovecii* prophylaxis).

In India, several isolates of *Salmonella* displaying a high level of resistance to azithromycin have been identified.[1] Paradoxically, there has been newfound susceptibility to older antibiotics (ampicillin, cotrimoxazole, chloramphenicol), leading to speculation about antibiotic recycling.[1] The trend toward an increasing emergence of extended beta-lactamases and strains resistant to azithromycin will greatly limit therapeutic options, leaving only carbapenems and tigecycline as secondary antimicrobial drugs.

The high prevalence of foodborne salmonellosis worldwide makes it a significant public health problem. Good food hygiene and practices are undoubtedly important in decreasing the rates of *Salmonella* infections. With the current ubiquitous antibiotic prescription practices, antimicrobial resistance patterns

will no doubt continue to evolve. At present, azithromycin remains a viable alternative to ciprofloxacin for the treatment of uncomplicated typhoid fever and invasive non-typhoidal salmonellosis in the community, but it must be used with care to prevent rapid development of widespread resistance. Further research is also needed to determine the clinically relevant breakpoints of azithromycin by reviewing the treatment outcome of azithromycin in relation to its MICs.

In conclusion, the emergence of azithromycin-resistant *Salmonella* is a worrying phenomenon. We advocate testing for azithromycin susceptibility in ciprofloxacin-resistant cases and high-risk groups, such as the immunocompromised (e.g. HIV patients) and travellers from endemic areas. In addition, local susceptibility data should be collected and considered when selecting empirical antibiotics for cases of suspected *Salmonella* infections.

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