

## Case Reports and Series

Azithromycin susceptibility testing of *Salmonella enterica* serovar Typhi: Impact on management of enteric fever

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## ABSTRACT

**Background:** Drug-resistant enteric fever is increasingly common in the Indian subcontinent. Correctly determining azithromycin resistance matters where drug-resistant enteric fever is common and oral therapy necessary.

**Case report:** In two patients returning from Pakistan to the UK with cephalosporin-resistant *Salmonella enterica* serovar Typhi, gradient strip testing erroneously indicated azithromycin resistance; the errors were detected by repeat testing and confirmed by whole genome sequencing.

**Results:** Both patients were treated with meropenem and, when revised susceptibility results were known, with azithromycin, allowing a switch to oral therapy.

**Conclusion:** As cephalosporin resistance becomes more common, azithromycin will be key for treating enteric fever and optimizing practice in susceptibility testing will be crucial. Practitioners should be aware of key steps to minimize error in azithromycin susceptibility testing, and should be alert for possible errors when reported azithromycin resistance is discordant with known prevalence of resistance.

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## Introduction

As extensively drug-resistant (XDR) strains of *Salmonella enterica* subsp. *enterica* serovar Typhi ("S. Typhi") have emerged, oral treatment recommendations for enteric fever have moved away from traditionally used antibiotics towards azithromycin (Chatham-Stephens et al., 2019). Testing the susceptibility of S. Typhi to azithromycin raises particular issues around interpretation of results and ensuring tests are appropriately controlled. In the two case descriptions that follow, we illustrate clinical contexts in which difficulties with azithromycin susceptibility testing may manifest. We then discuss the implications for testing and

treatment of enteric fever, and for future monitoring for emergence of antibiotic resistance.

## Case 1

A 46-year-old lady presented in January 2019 with a history of fever, right flank pain, dysuria, and diarrhoea following travel to Karachi and Lahore in Pakistan during December 2018 and early January 2019. She had a background of intentional purging and had taken laxatives in the preceding week, although diarrhoea began in Pakistan without taking laxatives. Fever began two days after return to the United Kingdom.

Examination demonstrated a temperature of 38 °C and right renal angle tenderness but was otherwise unremarkable. She was initially treated with ceftriaxone (2 g intravenously (IV) daily) and ciprofloxacin (500 mg orally (PO) once then 400 mg IV twice daily).

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Blood cultures taken at admission grew *S. Typhi* (identified by Matrix-Assisted Laser Desorption/Ionization-Time of Flight mass spectrometry and bioMérieux Analytical Profile Index-20E test; confirmed by specific antisera) on day 2 of admission. The isolate's susceptibility pattern was determined by MIC using bioMérieux Etest interpreted using European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint criteria (R = resistant, S = susceptible). The MIC in mg/L of ciprofloxacin was 1 [R], azithromycin 96 [R], ceftriaxone 32 [R], ertapenem 0.047 [S], and meropenem 0.06 [S]. MAST D68C test detected extended-spectrum beta-lactamase (ESBL) presence.

Antimicrobial therapy was switched on day 3 to ceftriaxone (2 g IV daily) and azithromycin (1 g PO once) following pathogen identification, then to meropenem (1 g 3 times daily) monotherapy following results of in-house antimicrobial susceptibility testing on day 4. Fever persisted until the switch to meropenem, then abated after 96 h. The C-reactive protein (CRP) remained 100 mg/L until meropenem commencement, then declined towards 5 mg/L after 12 days of meropenem; abdominal pain subsided with the CRP decline.

The isolate was sent to the Gastrointestinal Bacteria Reference Unit (GBRU), Public Health England, Colindale, United Kingdom, where identification as *S. Typhi* was confirmed by PCR and whole genome sequencing (WGS; sequence data at Sequence Read Archive accession [SRR8524701](#)), and MICs using bioMérieux Etest interpreted using EUCAST breakpoint criteria were determined (in mg/L) and reported as: ciprofloxacin 0.19 [R], azithromycin 8 [S], ceftriaxone >32 [R]. Genotypic resistance predictions were made for surveillance purposes (i.e. not reported clinically) from the WGS data using a previously described method ([Day et al., 2018](#)) that compares against reference sequences for acquired resistance genes curated from the Comprehensive Antimicrobial Resistance Database ([McArthur et al., 2013](#)) and the ResFinder datasets ([Zankari et al., 2012](#)). These WGS-based predictions confirmed the presence of genes conferring resistance to chloramphenicol (*catA1*), sulfamethoxazole (*sul1*), quinolones (*gyrA\_SET* [83:S-F]) and cephalosporins (CTX-M-15) and the absence of known genes conferring macrolide resistance (see [Table 1](#)).

Azithromycin (500 mg PO daily) was reintroduced on day 9, in addition to meropenem, following reference laboratory susceptibility testing results. Computed tomography of the abdomen and pelvis on day 11 showed mild appendicitis, which resolved with medical management. The patient was discharged on day 17, having received a cumulative total of 13 days of meropenem and 8 days of azithromycin, without follow-on therapy, and remained well at 3-week follow-up.

[Fig. 1](#) gives timing details of azithromycin susceptibility testing results and antimicrobial therapy.

## Case 2

A 6-year-old boy presented in January 2019 two to three days after returning from a two-week holiday in Karachi, Pakistan, with a history of fever, rigors, decreased appetite, vomiting, lethargy, not walking, and two days of offensive loose stool with abdominal pain. There was no blood or mucus in stool, rash, or headache. He had been managed in primary care for suspected viral illness. Past medical history was notable only for eczema.

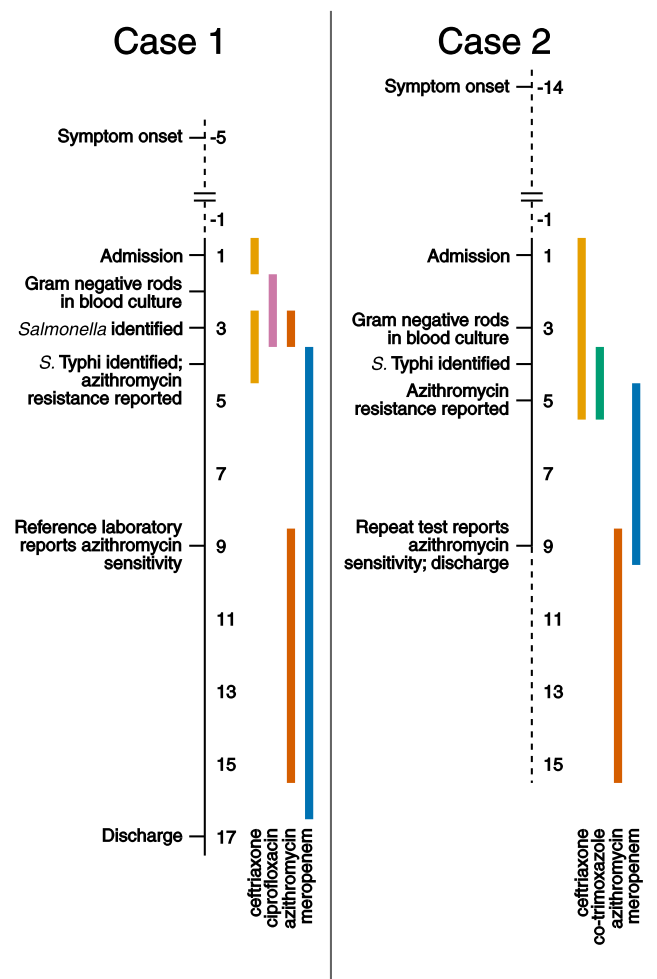
Examination demonstrated a temperature of 39.9 °C with a relative bradycardia, pallor, lethargy, and dry mucous membranes. He was treated with intravenous ceftriaxone (50 mg/kg daily for two days then 80 mg/kg daily for three days).

Blood cultures and stool culture taken at admission grew *S. Typhi* (identified by bioMérieux VITEK 2 system; confirmed by specific antisera). The isolate was, by disc testing, resistant to

**Table 1**

Summary of antimicrobial resistance genes identified in patient *S. Typhi* isolates by whole genome sequencing and a resistance prediction pipeline (× = absent, ✓ = present).

Antimicrobial	Gene	Case 1	Case 2
Chloramphenicol	<i>catA1</i>	✓	✓
Trimethoprim	<i>dfrA7</i>	×	✓
Sulphonamides	<i>sul1</i>	×	✓
	<i>sul2</i>	✓	✓
Beta-lactams	<i>blaTEM-1</i>	×	✓
	CTX-M-15	✓	✓
Aminoglycosides	<i>strA</i>	×	✓
	<i>strB</i>	×	✓
Fluoroquinolones	<i>gyrA_SET</i> [83:S-F]	✓	✓
	<i>qnrS1</i>	×	✓



**Fig. 1.** Timelines of major events in *S. Typhi* culture and azithromycin sensitivity testing, and of antimicrobial therapy administered, in the two cases.

ciprofloxacin, ceftriaxone and co-trimoxazole, but susceptible to meropenem. The azithromycin MIC using bioMérieux Etest was 32 mg/L [R]. Repetition in-house gave azithromycin MIC as 8 mg/L [S]. GBRU testing confirmed the identification as *S. Typhi* by PCR/WGS (Sequence Read Archive accession [SRR8489337](#)) and determined the isolate's azithromycin MIC by bioMérieux Etest to be 4 mg/L [S]; genotypic resistance predictions confirmed the presence of genes conferring resistance to chloramphenicol (*catA1*), trimethoprim (*dfrA7*), sulfamethoxazole (*sul1*[v] and *sul2*[v]), quinolones (*gyrA\_SET*[83:S-F] and *qnrS1*), ampicillin (*blaTEM-1*) and cephalosporins (CTX-M-15), and the absence of known genes conferring macrolide resistance (see [Table 1](#)).

**Table 2**Summary recommendations for azithromycin quality control (QC) in *S. Typhi* susceptibility testing from testing guidelines and protocols.

Guideline or protocol	bioMérieux Etest (2012)	CLSI (2019)	EUCAST (2019)
QC strain selection criterion	Antibiotic	Antibiotic	Clinical isolate organism
QC strain recommendation	"Appropriate quality control strains"; proprietary LyfoCults Plus organisms mentioned	Not explicitly stated; strains that have reference QC ranges determined are listed	<i>Escherichia coli</i> ATCC 25922*
QC frequency	"Should be established by the individual laboratory"	Each test day (some exceptions)	"[D]aily, or at least four times per week, for antibiotics that are part of routine panels"

\* Recommended for all Enterobacterales; not suitable for azithromycin QC owing to intrinsic resistance.

Initial ceftriaxone therapy was supplemented with cotrimoxazole (27 mg/kg IV twice daily) at first laboratory indication of cephalosporin resistance. Therapy was switched to meropenem (1 g 3 times daily) on day 5 of admission, and to azithromycin (250 mg PO daily) on day 9 following repeat antimicrobial susceptibility testing. Fever abated after 24 h of meropenem; the patient was discharged on azithromycin. He remained well at follow-up.

Fig. 1 again gives timing details of azithromycin susceptibility testing results and antimicrobial therapy.

## Discussion

With the advent of resistance to traditional first-line antibiotics for enteric fever, treatment with extended-spectrum cephalosporins and azithromycin has become important. In cases of ceftriaxone-resistant *S. Typhi*, carbapenems have been used, although carbapenem outcome data are limited and failure of meropenem monotherapy has been reported. (Blumentrath et al., 2019; Liu et al., 2020) Azithromycin can be given orally and is associated with lower relapse rates than ceftriaxone. (Trivedi and Shah, 2012)

*In vitro* azithromycin resistance has been described in typhoidal *Salmonella* isolates for 20 years, (Butler et al., 1999) with the first clinical treatment failure described in 2010. (Molloy et al., 2010) A recent Bangladesh study found a high proportion of azithromycin-resistant clinical isolates (31/33 *S. Typhi* isolates). (Ahsan and Rahman, 2019)

Ceftriaxone resistance was first reported in Bangladesh in 1999 (Saha et al., 1999) and cephalosporin resistance subsequently reported sporadically in the Indian subcontinent, (Bhattacharya et al., 2011; Qamar et al., 2014) in 2 instances with additional azithromycin resistance. (Munir et al., 2016; Patel et al., 2017) A large outbreak of cephalosporin-resistant *S. Typhi* was reported in Pakistan's Sindh province in 2018, mediated by a plasmid-encoded ESBL. (Klemm et al., 2018) These extensively drug-resistant (XDR) strains are resistant to first-line antibiotics plus extended-spectrum cephalosporins, leaving carbapenems and azithromycin the only established treatment options. Preserving azithromycin susceptibility here is crucial as is detecting early development of dual cephalosporin-azithromycin resistance, so a reliable local azithromycin susceptibility test is essential.

We describe two patients where local laboratories encountered difficulties testing azithromycin susceptibility in XDR *S. Typhi*, leading to clinical management changes. The reason for the erroneous results is unclear; however, these difficulties have been reported elsewhere (Goldblatt et al., 2020) and the potential for error in azithromycin susceptibility reporting is therefore an issue deserving of attention both by laboratory staff and by clinicians acting on the results of azithromycin susceptibility tests. Testing guidelines do not provide mechanisms to distinguish between possible causes of error.

Several factors can cause errors in antimicrobial susceptibility testing using gradient MIC strips, including mixed isolates, agar

depth and pH, gradient strip storage, inoculum level, incubation atmospheric conditions and temperature, and subjectivity in reading. Multiple manufacturers now produce azithromycin gradient MIC strips, and although in our cases the bioMérieux Etest was used, similar issues have been reported with strips from at least one other manufacturer (Goldblatt et al., 2020), indicating this is not merely an issue with one platform. We reiterate manufacturer- and guideline-recommended best practice to reduce testing strip degradation risk, discuss techniques to ensure uniformity and accuracy, plus emphasize appropriate control organism use to mitigate potential for undetected errors.

Strip degradation can be significant. Gradient strip storage must comply with manufacturers' instructions, avoiding use past expiry dates. Before opening, strips must reach room temperature, enabling moisture on packaging to evaporate. Strips in open packages must be protected from moisture, heat, and strong light exposure by resealing or placing into a desiccant-containing airtight container, and avoiding storage temperature variations (bioMérieux Etest, 2012).

Incubation set-up should be completed within 15 min of broth inoculation, with agar plates inverted in stacks not exceeding five plates and strict adherence to the 16–20 h incubation time. Having two separate readers interpreting and agreeing MIC results is good practice. Azithromycin is bacteriostatic: gradient strip manufacturers advise "trailing edge" (80% inhibition) use to determine its MIC (bioMérieux Etest, 2012).

Quality control (QC) guidelines focus on which antibiotics reference organisms can test, rather than on scenario-specific reference organism recommendations. One guideline recommends QC organisms by clinical isolate organism, not antibiotic, consequently recommending an azithromycin-inappropriate control organism. No testing guideline or protocol advocates testing a control organism on every occasion (Table 2). No guideline explicitly recommends *Staphylococcus aureus* for QC of *S. Typhi* azithromycin resistance testing, although experimental use has been described. (Sharma et al., 2019) We emphasize the importance of accompanying azithromycin susceptibility tests with testing a suitable QC strain (e.g. *S. aureus* ATCC 29213), using the same medium and device as clinical isolates. Where azithromycin resistance is uncommon, resistant isolates should be retested or referred to requesting clinicians. All *Salmonella* isolates should be referred to a reference laboratory for confirmatory testing where available.

We recommend these steps in laboratories undertaking *S. Typhi* azithromycin susceptibility testing, to ensure patients promptly receive correct, definitive antimicrobial therapy.

## CRedit authorship contribution statement

**Jordan P. Skittrall:** Conceptualization, Investigation, Resources, Writing - original draft, Project administration. **David Levy:** Investigation, Resources, Writing - original draft. **Christian Obichukwu:** Investigation, Resources, Writing - original draft. **Amy Gentle:** Investigation, Resources. **Marie A. Chattaway:** Conceptualization,

Investigation, Resources. **David Hayns:** Investigation, Resources. **Clare Etheridge:** Investigation, Resources. **Christopher M. Parry:** Conceptualization, Supervision. **Vanessa Wong:** Conceptualization, Supervision. **James Whitehorn:** Conceptualization, Supervision.

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## Disclosures

J.W. reports personal fees from Janssen Pharmaceuticals. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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