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Original Article

First report of ciprofloxacin-resistant *Salmonella* Enteritidis isolated from human cases in Morocco

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Abstract

Introduction: *Salmonella enterica* serovar Enteritidis is a principal cause of foodborne and invasive infections worldwide. Ciprofloxacin continues to be a primary treatment for invasive cases, but resistance has emerged in several regions. In Morocco, although *S.* Enteritidis is frequently isolated, no ciprofloxacin-resistant isolates have been reported to date.

Methodology: A total of 60 *Salmonella* strains were detected in clinical samples from the Mohammed VI University Hospital Center in Marrakech between 2018 and 2024. Strain S54 was identified as *S.* Enteritidis by serotyping. Antimicrobial susceptibility testing was performed by disk diffusion. Whole-genome sequencing was conducted using Illumina technology, and resistance and virulence determinants were analyzed with ResFinder, PlasmidFinder, and VFDB databases.

Results: Three isolates (5%), including the S54 strain, showed resistance to ciprofloxacin. Genomic analysis identified a D87Y substitution in *gyrA* and the presence of efflux genes (*mdsA, mdsB*). Several virulence genes, including those located in SPI-1 and SPI-2, were also detected.

Conclusions: This study represents the first report of a ciprofloxacin-resistant *S.* Enteritidis strain in Morocco. These findings highlight the urgent need to strengthen molecular surveillance and implement preventive measures against fluoroquinolone-resistant *Salmonella*.

Key words: *S. Enteritidis*; ciprofloxacin; resistance; whole-genome sequencing.

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Introduction

Salmonella species are one of the most important zoonotic foodborne pathogens worldwide, contributing to high levels of morbidity and mortality [1]. Globally, it is a leading cause of bacterial gastroenteritis, responsible for an estimated 93.8 million cases and 155,000 deaths annually [2]. Among more than 2,500 known serotypes, *Salmonella enterica* serotype Enteritidis (SE) remains the most frequently reported worldwide [1].

Antibiotics are the primary treatment for invasive infections of *Salmonella* strains. Currently, ciprofloxacin and ceftriaxone are the drugs of choice for treating severe or systemic salmonellosis [2]. However, the widespread use of fluoroquinolones has driven the global emergence of resistant strains in both human and animal isolates, particularly S. Enteritidis [3].

Fluoroquinolone resistance in *Salmonella enterica* is mostly due to chromosomal mutations in the quinolone resistance-determining regions (QRDR) of the *gyrA*, *gyrB*, *parC*, and *parE* genes, particularly at codons 83 and 87 in *gyrA*, and at codon 80 in *parC* [4].

In Morocco, *S.* Enteritidis is the most frequently isolated serotype [5,6]. Reduced susceptibility or intermediate resistance of S. Enteritidis to ciprofloxacin has been previously reported in Morocco [7]. However, molecular data on the resistance mechanisms of Moroccan isolates remain scarce. To date, no fully ciprofloxacin-resistant isolate has been confirmed by genomic analysis. Here, we describe the first ciprofloxacin-resistant S. Enteritidis strain from a human clinical sample, and the aims of this work were to characterize antimicrobial resistance and virulence genes of this strain by whole-genome sequencing

Table 1. Antibiotic resistance profile of Salmonella Enteritidis isolate S54.

Strain	Serovar	Antibiotic Resistance Profile													
		AMP	AMC	CTX	CAZ	CRO	FEP	PTZ	ETP	IPM	SXT	NOR	CIP	GMN	AKN
S54	*S. Enteritidis*	S	S	S	S	S	S	S	S	S	S	S	R	R	R

S: Susceptible; R: Resistant; AMP: Ampicillin; AMC: Amoxicillin/clavulanic acid; CTX: Cefotaxime; CAZ: Ceftazidime; CRO: Ceftriaxone; FEP: Cefepime; PTZ: Piperacillin/tazobactam; ETP: Ertapenem; IPM: Imipenem; NOR: Norfloxacin; CIP: Ciprofloxacin; GMN: Gentamicin; AKN: Amikacin; and SXT: Trimethoprim/sulfamethoxazole.

(WGS) and to study phenotypic antimicrobial resistance patterns of Moroccan *Salmonella* strains. Given the critical role of ciprofloxacin and the high prevalence of *S.* Enteritidis in Morocco, these findings underscore the urgent need to strengthen molecular surveillance.

Methodology

Bacterial isolation, serotyping, and antimicrobial susceptibility testing

From January 2018 to June 2024, a total of 60 *Salmonella* strains were isolated from clinical samples following REMIC (2022) guidelines.

All *Salmonella* isolates were identified using matrix-assisted laser desorption/ionization Time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics, Billerica, MA, USA). Antimicrobial susceptibility testing was carried out using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Bio-Rad, Marnes-la-Coquette, France). Serotyping of the *Salmonella* isolates was conducted using slide agglutination with O, H, and Vi antigen-specific antisera obtained from the Institute Pasteur Morocco, and interpreted according to the Kauffmann White classification scheme.

Genome Sequencing and Bioinformatic Analysis

Genomic DNA was extracted using Qiagen DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. Libraries were prepared using the Nextera XT DNA library preparation kit (Illumina, San Diego, CA, USA) and sequenced using the Illumina NextSeq 500 platform. After demultiplexing and removal of adapters, reads were trimmed and filtered with fastp v0.22.0 using default options.

Quality control of the raw and filtered reads was assessed using FastQC v0.11.9. De novo genome assembly was performed with SPAdes v3.15.5, and contigs shorter than 2500 bp were removed using BBMap v39.01. Genome completeness and contamination were evaluated with CheckM v1.2.2. Annotation was conducted using Prokka v1.14.6.

Antimicrobial resistance and virulence genes were identified using ABRicate v1.0.1 with the VFDB, ResFinder, and PlasmidFinder databases, as well as with AMRFinderPlus v3.11.4 using the NCBI-AMR database. *In silico* serotyping was performed using SeqSero2, which predicts the serovar based on the antigenic formula.

Results

Among the 60 *Salmonella* strains identified, 5% (n = 3) were resistant to ciprofloxacin. These included two *S.* Typhimurium and one *S.* Enteritidis (S54). The latter was isolated in 2024 from a pus sample collected at the Infectious Diseases Unit of the Mohammed VI University Hospital Center in Marrakech. The sample originated from a 24-year-old female patient hospitalized with symptoms of gastrointestinal infection, including fever, diarrhoea, and vomiting.

Serotyping confirmed strain S54 as *Salmonella enterica* serovar Enteritidis. Antimicrobial susceptibility testing revealed resistance to ciprofloxacin, amikacin, and gentamicin (Table 1). Whole-genome sequencing of strain S54 (Illumina NextSeq 500) revealed resistance genes, mutations, and virulence factors. Key features are shown in Tables 2 and 3, and the genome is available in GenBank (Accession number JBQPFZ000000000).

Table 2. Antimicrobial resistance genes and mutations in strain S54.

Resistance Gene	Class	Length (bp)	Match full name	Protein length	% Identity	Mutation	AA Change (Isolate → Reference)
mdsB	EFFLUX	3164	multidrug efflux RND transporter permease subunit MdsB	1055	99.81		Threonine → Alanine
mdsA	EFFLUX	1223	multidrug efflux RND transporter periplasmic adaptor subunit MdsA	408	98.28		Multiple substitutions EX: Threonine → Alanine Valine → Isoleucine Serine → Proline Leucine → Phenylalanine
*gyrA*_D87Y	QUINOLONE	2633	*Salmonella* quinolone resistant GyrA	878	99.89	D87Y	Aspartic acid → Tyrosine

Table 3. Virulence genes detected in strain S54.

Virulence Gene	Function	Length (bp)	Protein length	% Identity to reference
sinH	intimin-like inverse autotransporter SinH.	1931	644	99.84
sseK3	type III secretion system effector arginine glycosyltransferase SseK3.	1004	335	100.00
lpfB	long polar fimbrial chaperone LpfB.	695	232	100.00
spvD	SPI-2 type III secretion system effector cysteine hydrolase SpvD.	647	216	99.07
gtgA	type III secretion system effector protease GtgA.	683	228	99.56
sodC1	superoxide dismutase [Cu-Zn] SodC1.	530	177	100.00
sspH2	SPI-2 type III secretion system effector E3 ubiquitin transferase SspH2.	2363	788	99.11
avrA	type III secretion system YopJ family effector AvrA.	902	301	99.67
invA	type III secretion system export apparatus protein InvA.	2054	685	100.00

Discussion

A ciprofloxacin-resistant *Salmonella enterica* serovar Enteritidis strain (S54) was isolated from a clinical sample in Morocco and represented the first documented case in the country. Although this observation is limited to a single case, this resistance is alarming due to its invasive origin and clinical implications, as well as previous reports of reduced susceptibility without confirmed resistance [7]. The global emergence of fluoroquinolone-resistant *Salmonella enterica* presents a serious challenge to public health. Ciprofloxacin remains one of the first-line drugs of choice for the treatment of invasive *Salmonella* infections. However, the rate of resistance to this antibiotic has increased in recent years in both clinical and foodborne isolates worldwide [8].

The World Health Organization (WHO) listed fluoroquinolone-resistant *Salmonella* spp. among the priority pathogens for which new antibiotics are urgently required [2].

This resistance mainly results from mutations in quinolone resistance-determining regions (QRDR), especially in *gyrA*, and from plasmid-mediated genes such as *qnr*, *aac(6')-Ib-cr*, and *oqxAB* [2].

Alarmingly, the global emergence of ciprofloxacin-resistant *S.* Enteritidis has been increasingly reported in recent years, reflecting a concerning trend in both human and animal isolates. Studies from multiple regions, including Ghana, Poland, China, and Thailand, have documented a rising incidence of such resistant strains, indicating that this issue is widespread and not confined to a specific geographic area [3, 9-11].

The progressive global spread of these resistant strains highlights the urgent need for improved surveillance systems and the development of alternative therapeutic strategies. *S.* Enteritidis is among the most frequently reported serovars associated with human salmonellosis and has shown rising levels of ciprofloxacin resistance in both clinical and foodborne settings worldwide. This trend reinforces its status as a major public health issue, especially when resistance is associated with invasive infections [10].

The ciprofloxacin-resistant *S.* Enteritidis strain (S54) displayed a D87Y substitution in the *gyrA* gene, encoding the A subunit of DNA gyrase, an essential enzyme for DNA replication and maintenance. Mutations within the quinolone resistance-determining region (QRDR), especially at codons 83 and 87, are recognized to give resistance to fluoroquinolones. The D87Y alteration reduces ciprofloxacin binding affinity, leading to high levels of resistance [12]. Despite the absence of plasmid-mediated quinolone resistance (PMQR) genes in this strain, the presence of *mdsA* and *mdsB* efflux genes is known to contribute to multidrug resistance and to enhance survival under stress conditions in *Salmonella* species [13].

Similar profiles have been observed in clinical *S.* Enteritidis isolates from Ghana and Poland, where *gyrA* mutations and efflux mechanisms are commonly the main cause of fluoroquinolone resistance [14]. These findings indicate that the resistance profile of strain S54 corresponds with documented global mechanisms.

The invasive potential of strain S54 was inferred through the detection of virulence genes in the *Salmonella* Pathogenicity Islands SPI-1 and SPI-2, together with effectors such as *invA*, *sspH2*, and *SpvD*. SPI-1 promotes epithelial cell invasion through a type III secretion system, while SPI-2 supports intracellular survival and systemic dissemination. Together, these pathogenicity islands are essential factors in *Salmonella* virulence [15].

The effector *SpvD* contributes to virulence by interfering with host immune signaling through inhibition of the *NFkB* [16].

These findings suggest that S54 presents a dual threat through multidrug resistance and high potential for systemic spread and severe clinical outcomes.

Conclusions

This report represents the first ciprofloxacin-resistant *S.* Enteritidis case in Morocco, highlighting an alarming emergence in the country's antimicrobial resistance profile. The identification of a *gyrA* D87Y

mutation, efflux-associated resistance genes, and multiple virulence factors emphasizes the pathogenic potential of this strain.

These findings emphasize the necessity to enhance molecular surveillance for monitoring resistance, as well as to investigate novel therapeutic strategies and preventive measures against ciprofloxacin-resistant *Salmonella*. Further research on alternative antimicrobials, combination treatments, and novel strategies will be crucial for the optimal management of clinical cases and for the efficacy of antibiotics in treating invasive infections.

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Availability of data and materials

The datasets generated and analyzed during the present study are included in the body of this paper.

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Conflict of interest

No conflict of interest is declared.

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