The Emergence of Reduced Ciprofloxacin Susceptibility in Salmonella enterica Causing Bloodstream Infections in Rural Ghana

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Background. Salmonella ranks among the leading causes of bloodstream infections in sub-Saharan Africa. Multidrug resistant typhoidal and nontyphoidal Salmonella (NTS) isolates have been previously identified in this region. However, resistance to ciprofloxacin has rarely been reported in West Africa. This study aims to assess susceptibility against ciprofloxacin in Salmonella causing invasive bloodstream infections among children in rural Ghana.

Methods. From May 2007 until May 2012, children attending a rural district hospital in central Ghana were eligible for recruitment. Salmonella enterica isolated from blood cultures were assessed for ciprofloxacin susceptibility by Etest (susceptible minimum inhibitory concentration [MIC] ≤ 0.06 µg/mL). The gyrA, gyrB, parC, and parE genes were sequenced to identify mutations associated with changes in susceptibility to fluoroquinolones.

Results. Two hundred eighty-five Salmonella enterica isolates from 5211 blood cultures were most commonly identified as Salmonella enterica serovar Typhimurium (n = 129 [45%]), Salmonella enterica serovar Typhi (n = 89 [31%]), Salmonella enterica serovar Dublin (n = 20 [7%]), and Salmonella enterica serovar Enteritidis (n = 19 [7%]). All S. Typhi and S. Dublin were susceptible to ciprofloxacin. Reduced susceptibility (MIC >0.06 µg/mL) was found in 53% (10/19) of S. Enteritidis isolates and in 2% (3/129) of S. Typhimurium isolates. Sequencing detected a single gyrB mutation (Glu466Asp) and a single gyrA mutation (Ser83Tyr) in all 3 S. Typhimurium isolates, while 9 of 10 S. Enteritidis harbored single gyrA mutations (Asp87Gly, Asp87Asn, or Asp87Tyr). No mutations were found in the parC and parE genes.

Conclusions. Ciprofloxacin susceptibility in invasive NTS in rural Ghana is highly dependent on serotype. Although reduced ciprofloxacin susceptibility is low in S. Typhimurium, more than half of all S. Enteritidis isolates are affected. Healthcare practitioners in Ghana should be aware of potential treatment failure in patients with invasive S. Enteritidis infections.

Keywords. Africa; Ghana; Salmonella; susceptibility; multidrug resistant.

Invasive salmonellosis constitutes a significant disease burden worldwide, with an estimated 22 million cases of typhoid fever and 94 million cases of nontyphoidal Salmonella (NTS) infections each year [1, 2]. Both Salmonella enterica serovar Typhi and NTS serovars are known to cause invasive bacterial disease in sub-Saharan Africa, accounting for an estimated 10% and 17% of bacterial bloodstream infections, respectively [3]. A previous study in Ghana reported yearly cumulative incidences for children aged <5 years of 25 per 1000 for NTS and 3.3 per 1000 for S. Typhi bloodstream infections [4]. In addition, high case fatality rates for NTS have been reported in sub-Saharan Africa, ranging from 22% to 47% [5, 6]. Case fatality rates are predicted to increase further when affordable and locally available antimicrobial drugs become less effective. Resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (SXT) is increasing in sub-Saharan Africa and already leads to the use of more expensive antimicrobials, such as fluoroquinolones and third-generation cephalosporins [6–8]. High rates of multidrug resistant (MDR) S. Typhi have been observed in India (97%), Cambodia (90%), Iraq (81%), and Egypt (36%) and NTS observed in Cambodia (33%) [9–11].

A lower ciprofloxacin susceptibility breakpoint for S. Typhi and NTS (≤0.06 µg/mL) has recently been introduced by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee for Antimicrobial Susceptibility Testing [12]. Three studies from Ghana, conducted between 2001 and 2009, applied the previous breakpoint definition and found only ciprofloxacin-susceptible Salmonella isolates [4, 13, 14]. However, previous reports on ciprofloxacin resistance must be interpreted with caution, as their figures probably underestimate the actual antibiotic resistance situation. More recent

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The aim of this study was to measure ciprofloxacin susceptibility in Salmonella isolates from pediatric patients with bloodstream infections in rural Ghana and to characterize the underlying resistance mechanisms.

METHODS

Study Site and Study Population

The study was conducted at the Agogo Presbyterian Hospital, a district hospital with 250 beds, situated in the Asante Akim North municipality of the Ashanti Region in Ghana. All children <15 years of age admitted to this hospital were enrolled in this study; in the case a blood culture was performed, informed consent was provided. Children presenting with dermatological diseases and undergoing surgery were excluded from the study. From September 2007 until July 2009 and from February 2010 until May 2012, recruitment took place on the pediatric ward.

Ethical Considerations

The Committee on Human Research, Publications and Ethics, School of Medical Science, Kwame Nkrumah University of Science and Technology in Kumasi, Ghana, provided ethical approval for this study. All participants were informed about the study’s purpose and procedures. Written informed consent was obtained from the parents or the guardian on behalf of the study children prior to study enrolment.

Detection and Identification of Pathogens

Three milliliters of blood was drawn from each child, inoculated into a pediatric blood culture bottle (BACTEC Peds Plus/F, Becton Dickinson), and processed using a BACTEC 9050 blood culture system (Becton Dickinson) according to the manufacturer’s instructions. In case of bacterial growth, subcultures were performed on Columbia blood agar, chocolate agar, and McConkey agar (all Oxoid, Basingstoke, United Kingdom). Bacterial colonies were further identified by standard biochemical methods. Environmental bacteria and bacteria belonging to the skin flora (eg, coagulase-negative staphylococci, Corynebacterium species, and Bacillus species) were considered to be contaminants. Suspected Salmonella isolates were identified biochemically by API 20E tests (bioMérieux, Marcy L’Etoile, France). Typing was performed using multiplex polymerase chain reaction (PCR) for S. Typhi [15], Salmonella enterica serovar Typhimurium [15], Salmonella enterica serovar Enteritidis [16], and Salmonella enterica serovar Dublin [17] as described within this supplement [18]. The other Salmonella isolates were serotyped following the White–Kauffmann–Le Minor scheme at the German National Reference Centre for Salmonella (Robert Koch Institute, Wernigerode) [19].

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing against ampicillin, amoxicillin/clavulanic acid, chloramphenicol, SXT, ciprofloxacin, and ceftriaxone was performed for all Salmonella isolates by the disk diffusion method according to the 2013 CLSI M100-S23 guidelines (www.clsi.org). Ciprofloxacin minimum inhibitory concentrations (MICs) were determined by Etest (Oxoid). Isolates were interpreted as ciprofloxacin susceptible with an MIC ≤ 0.06 µg/mL, as intermediate (reduced susceptibility) with an MIC < 1 µg/mL and >0.06 µg/mL, and as resistant with an MIC ≥ 1 µg/mL. Salmonella isolates exhibiting resistance to ampicillin, SXT, and chloramphenicol were defined as MDR.

Sequencing of gyrA, gyrB, parC, and parE Genes

All isolates were stored at ~80°C and further processed at the Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany. DNA of all Salmonella isolates with a ciprofloxacin MIC ≥ 0.06 µg/mL was extracted using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Subsequently, the gyrA, gyrB, parC, and parE genes were amplified as previously described [20], and PCR amplicons were purified and sequenced by Eurofins (Hamburg, Germany). Forward and reverse sequences were assembled using the Seqscape Software version 2.1.1 (Applied Biosystems). Retrieved sequences were screened for mutations in comparison to the National Center for Biotechnology Information GenBank reference sequences with the accession numbers AE006468.1 (gyrA), Y07916.1 (gyrB), AE006468.1 (parC), and AE006468.1 (parE).

Presence of qnrA, qnrB, qnrS, and qepA

Multiplex PCR was performed [21] to assess the presence of the plasmid-mediated quinolone resistance determinants in qnrA, qnrB, qnrS [22], and qepA [23].

Efflux Pump Activity

All nonsusceptible Salmonella isolates were tested for the presence of efflux pump activity against ciprofloxacin. MICs were determined by broth microdilution method in presence and absence of the efflux pump inhibitor phenylalanine arginine β-naphthylamide (PAβN). A minimum 4-fold reduction in MIC in the presence of PAβN was regarded as evidence for increased efflux pump activity [24].

Epidemiological Analysis

Blood cultures obtained from the same patient within a 3-month period containing Salmonella isolates belonging to the same serovar were excluded from the analysis. Categorical variables were described as frequencies and expressed as percentages. Age was described using median and interquartile range (IQR). All data analyses were performed with stata software version 12 (StataCorp, College Station, Texas).

RESULTS

During the study period, 5211 blood cultures were performed from 4694 patients. Study participants had a median age of 24 months (IQR, 8–55 months) and 54.1% (n = 2541) were

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male. Out of 943 positive blood culture isolates, 521 contained a bacterial pathogen. The most frequently identified bacteria were NTS (38% \( n = 196 \)) and S. Typhi (17% \( n = 89 \)), followed by Staphylococcus aureus (13% \( n = 69 \)) and Streptococcus pneumoniae (12% \( n = 62 \)). Twenty-four NTS were missing and their serovars were not determined. Seven different serovars of NTS were detected, of which S. Typhimurium (54% \( n = 129 \)), S. Dublin (7% \( n = 20 \)), and S. Enteritidis (7% \( n = 19 \)) were the most common (Figure 1).

Resistance to ampicillin, chloramphenicol, and SXT was detected in 70%, 76%, and 76% of all S. Typhi isolates and in 76%, 70%, and 70% of NTS isolates, respectively. MDR was identified in 59 (66%) S. Typhi and 126 (67%) of NTS isolates. MDR rates among the NTS serovars were 5% (1/19) in S. Dublin, 11% (2/19) in S. Enteritidis, and 84% (108/129) in S. Typhimurium (Figure 2).

Complete resistance against ciprofloxacin—that is, an MIC \( \geq 1 \mu g/mL \) as defined by CLSI guidelines (2012)—was not observed in any of the Salmonella isolates. However, 10 (53%) S. Enteritidis and 3 (2%) S. Typhimurium isolates exhibited reduced susceptibility (ie, MICs between >0.06 \( \mu g/mL \) and \(<1 \mu g/mL \)). The majority of Salmonella strains with reduced susceptibility to ciprofloxacin were isolated between 2010 and 2012 (8% \( n = 11/138 \)), and lower numbers were observed between 2007 and 2009 (1% \( n = 2/150 \)).

Nine of the 10 S. Enteritidis strains with reduced susceptibility to ciprofloxacin had a single base-pair mutation in the gyrA gene at codon 83 (Asp to Tyr) in comparison to a susceptible wild-type. Among the 3 S. Typhimurium isolates with reduced susceptibility to ciprofloxacin, single base-pair mutations occurred at codon 83 (Ser to Tyr) in the gyrA gene and at codon 466 (Glu to Asp) in the gyrB gene (Table 1). Notably, S. Typhi, S. Dublin, and other less commonly isolated Salmonella serovars did not exhibit reduced ciprofloxacin susceptibility (Figure 2).

A total of 272 (95.4%) Salmonella isolates were categorized as susceptible to ciprofloxacin. However, 21 (7.4%) isolates had an MIC against ciprofloxacin of 0.06 \( \mu g/mL \), just below the

### Table 1. Salmonella enterica Isolates With Ciprofloxacin Minimum Inhibitory Concentrations \( \geq 0.015 \mu g/mL \) and Corresponding Target Mutations or Increased Efflux Pump Activities

<table>
<thead>
<tr>
<th>Salmonella enterica Serovars (No.)</th>
<th>Mutation/Efflux Pump (No.)</th>
<th>Amino Acid Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin MIC = 0.015–0.03 ( \mu g/mL )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. Enteritidis (9)</td>
<td>None (0)</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin MIC = 0.06 ( \mu g/mL )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. Dublin (11)</td>
<td>None (0)</td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin MIC = 0.12–0.25 ( \mu g/mL )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. Enteritidis (10)</td>
<td>Efflux pump (1)</td>
<td></td>
</tr>
<tr>
<td>S. Typhimurium (3)</td>
<td>GyrA-87 (2)</td>
<td>GAC (Asp) to TAC (Tyr)</td>
</tr>
<tr>
<td>S. Typhimurium (3)</td>
<td>GyrA-87 (1)</td>
<td>GAC (Asp) to TAC (Tyr)</td>
</tr>
<tr>
<td>S. Typhimurium (3)</td>
<td>GyrA-87 (2)</td>
<td>GAC (Asp) to TAC (Tyr)</td>
</tr>
<tr>
<td>S. Typhimurium (3)</td>
<td>GyrB-466 (1)</td>
<td>GAA (Glu) to GAT (Asp)</td>
</tr>
</tbody>
</table>

Abbreviations: Asn, asparagine; Asp, aspartic acid; Glu, glutamic acid; Gly, glycine; MIC, minimum inhibitory concentration; No., frequency; Ser, serine; Tyr, tyrosine.
susceptibility breakpoint. Of those, 1 S. Typhi isolate had a single base-pair mutation in the gyrA gene, which changed a glutamic acid to glycine at codon 133. No mutations were observed in the parC and parE genes in any of the isolates. The transferable resistance genes qnrA, qnrB, qnrS, and qepA were not detected in nonsusceptible isolates.

A 16-fold increase in ciprofloxacin susceptibility (MIC = 0.004 µg/mL) has been determined for 1 S. Enteritidis isolate lacking a mutation in genes gyrA, gyrB, parC, or parE in the presence of the efflux pump inhibitor PAβN (Table 1). In the absence of PAβN, the ciprofloxacin MIC of that isolate was 0.064 µg/mL by broth dilution method. This is indicative of an increased activity of a major MDR efflux pump, potentially AcrAB-TolC. However, this does not contribute to a significant increase in ciprofloxacin resistance. In all other isolates of S. Enteritidis and S. Typhimurium, a 2- to 4-fold decrease in MIC of ciprofloxacin was detectable, which points to a basic efflux activity in all isolates without significant impact on ciprofloxacin susceptibility.

**DISCUSSION**

This study confirms the emergence of *S. enterica* strains with reduced susceptibility to ciprofloxacin in West Africa. In 3 previous studies from Ghana, conducted between 2002 and 2009, a total of 170 NTS and 113 S. Typhi were isolated from bloodstream infections; only 2 NTS isolates were identified with reduced susceptibility to ciprofloxacin [4, 13]. However, the *Salmonella* serovars of these strains were not specified. In the study presented here, 6.6% of all NTS isolates showed reduced ciprofloxacin susceptibility. Possible explanations are a true increasing prevalence of reduced ciprofloxacin-susceptible strains or the application of lower breakpoints as recommended by the most recent CLSI guidelines. Nevertheless, 85% of the isolates with reduced ciprofloxacin susceptibility in this study were isolated in the last 3 years of the 6-year study period, which suggests a gradual increase of ciprofloxacin-resistant invasive *Salmonella* in rural Ghana since 2007. This is in agreement with a review article by Reddy et al, which did not report any ciprofloxacin-resistant S. Enteritidis and S. Typhimurium organisms isolated from bloodstream infections across 7 African countries between 1993 and 2006 [3]. An additional review article, which focused on Central Africa, concluded that fluoroquinolone resistance had emerged in NTS organisms from 1999 onward [25]. More recently, between 2007 and 2010, 4.3% of NTS from bloodstream infections in the Democratic Republic of Congo presented with reduced susceptibility to ciprofloxacin [26].

Ciprofloxacin-resistant S. Typhi was not observed in the current study. Nevertheless, 1 S. Typhi isolate with an MIC of 0.06 µg/mL and a mutation in the gyrA gene (Glu133Gly) was detected. Studies from South Africa and East Africa have already reported emerging ciprofloxacin resistance of S. Typhi; thus, an increase could well occur in West Africa in the near future [27, 28]. Notably, strains with reduced susceptibility to ciprofloxacin were unevenly distributed among serovars in the present study, with a high proportion in S. Enteritidis (55%) compared with S. Typhimurium (2%) and S. Dublin (0%). The spread of successfully emerging MDR genotypes from and within Africa has been described recently for S. Kentucky and S. Typhimurium (ST313) [29, 30]. Whether these S. Enteritidis isolates with reduced susceptibility have a distinct genotype needs to be assessed and monitored in future studies.

With the exception of 1 isolate, all mutations identified were located in the *gyrA* gene, at codons 83 and 87, which are the most commonly reported mutations worldwide [26, 31]. As seen in the present study, single *gyrA* mutations typically confer low-level resistance to ciprofloxacin. Mutations in the *gyrB* gene remain rare. The mutation at codon Glu466 of *gyrB* observed in this study has not been described so far; however, the association of this mutation with reduced ciprofloxacin susceptibility is described in this supplement [32]. The mutation at codon Glu133 in S. Typhi has been shown previously in ciprofloxacin-resistant isolates, but only in combination with a second mutation in *gyrA* or *gyrB* [33]. High proportions of MDR *Salmonella* as seen in this study have been reported from various African countries. In case of increasing ciprofloxacin resistance, in particular in S. Enteritidis, ceftriaxone remains the only efficient and available antibiotic in this region [5, 7, 26].

The study is limited to 2 different recruitment periods; both took place on the pediatric ward of the same hospital. In general, hospital attendees might show higher antimicrobial resistance rates than people without access to health services, because they have a higher probability of previous antimicrobial treatment and may be exposed to infectious agents circulating within hospitals, which are more likely to be resistant against standard antimicrobials [34]. Plasmid-mediated quinolone resistance determinants (eg, qnrA, qnrB, qnrS, and qepA) were not detectable in the isolates. A contribution of a deregulated MDR efflux pump as resistance mechanism, apart from those described, cannot be ruled out.

**CONCLUSIONS**

This study confirms the presence of NTS isolates with reduced susceptibility to ciprofloxacin in Ghana, whereas S. Typhi remains susceptible to antimicrobials commonly used in the clinical setting. In the context of other studies in Africa, gradually decreasing ciprofloxacin susceptibility is apparent. Given that the majority of isolates are already resistant to ampicillin, chloramphenicol, and cotrimoxazole, this development might threaten effective patient management in the near future. For S. Enteritidis, this has already become reality in the study region.

**Notes**

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