Increasing antimicrobial resistance in nontyphoid Salmonella species has been a serious problem for public health worldwide. The high rate of resistance is hampering the use of conventional antibiotics, and growing resistance to newer antimicrobial agents is aggravating the situation. The circumstances of occurrence and spread of antimicrobial resistance are complex; however, a major cause is the widespread use of antimicrobial agents in food animals, particularly in animal feed. Genetic analysis has indicated that the source of resistance is frequently a transferable plasmid. Recent studies have revealed that some serotype-specific virulence plasmids form hybrid plasmids through recombination with resistance plasmids or acquire gene cassettes consisting of multiple resistance genes. Such evolutionary events provide a virulent strain the advantage of survival in an unfavorable drug environment. In view of the serious implications associated with drug-resistant Salmonella species, a more deliberate use of antibiotics in both human medicine and animal industry is warranted. Continued surveillance of antimicrobial resistance and use of antimicrobial agents in food animals is also indispensable.

Salmonella enterica is a major pathogen in humans as well as in animals and comprises >2000 serotypes. They are widely dispersed in nature and are common inhabitants of the intestinal tract of domesticated and wild mammals, reptiles, birds, and even insects. The highly adapted S. enterica Typhi causes typhoid fever only in humans, whereas other serotypes, namely nontyphoid Salmonella serotypes, can cause a wide spectrum of diseases in humans and animals, such as acute gastroenteritis, bacteremia, and extraintestinally localized infections involving many organs. Although intestinal infection caused by nontyphoid Salmonella serotypes is usually self-limiting, effective antimicrobial therapy is essential if spread beyond the intestine occurs. In the past 2 decades, there have been many reports addressing the deteriorating situation of the increasing resistance to medically important antimicrobial agents among nontyphoid Salmonella serotypes. The present review aims to provide information on the current situation of antimicrobial resistance, the associated resistance mechanisms of fluoroquinolones and extended-spectrum cephalosporins, the mode of spread, and the impact on clinical medicine.

CURRENT STATUS OF ANTIMICROBIAL RESISTANCE IN NONTYPHOID SALMONELLA SEROTYPES

Antimicrobial resistance in nontyphoid Salmonella serotypes has been a global problem. Surveillance data demonstrated an obvious increase in overall antimicrobial resistance among salmonellae from 20%–30% in the early 1990s to as high as 70% in some countries at the turn of the century (table 1). The resistance rate, however, varies with different serotypes and different antibiotics. S. enterica Typhimurium DT104 S. Typhimurium was isolated and found to be simultaneously resistant to 5 antimicrobial agents (ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline) [10]. In the United Kingdom, S. Typhimurium DT104 had become the second most prevalent strain of Salmonella isolated from humans, following S. Enteritidis strain PT4, in the mid-1990s [11]. In the United States, the prevalence of S. Typhimurium isolates
that are concurrently resistant to these 5 drugs increased from <1% in 1979–1980 to 34% in 1996; most of the isolates were DT104 and showed 1 predominant genotype, indicating clonal spread [6].

Because of the increased resistance to conventional antibiotics, extended-spectrum cephalosporins and fluoroquinolones have become the drugs of choice for the treatment of infections caused by multidrug-resistant Salmonella serotypes. However, since 1991, many countries have been reporting outbreaks or cases of infections due to salmonellae that were resistant to extended-spectrum cephalosporins [12, 13]. In 2000, a multiple–health care center survey in 10 European countries identified a cefotaxime resistance rate of 0.6% in Salmonella isolates recovered from human sources [14]. Such a trend of increase in drug-resistant salmonellae was also noted in some Asian countries, including Taiwan (table 1).

Resistance to other antibiotics—notably, resistance to quinolones (e.g., nalidixic acid) and their derivatives, such as fluoroquinolones (e.g., ciprofloxacin)—has also begun to emerge. In the United States, a national survey conducted in 1994–1995 found that, of the 4008 Salmonella isolates tested, 21 (0.5%) were resistant to nalidixic acid and 1 (0.02%) was resistant to ciprofloxacin (MIC, ≥4 μg/mL); by 2000, the rate of nalidixic acid resistance had increased 5-fold, to 2.5% [15]. In the United Kingdom, reduced ciprofloxacin susceptibility (MIC, ≥0.25 μg/mL) in Salmonella isolates increased from 0.3% in 1991 to 2.1% in 1994 and to ~5% in 1996 [5]. Of particular concern is an outbreak of infection with quinolone-resistant S. Typhimurium DT104 that has spread from animals to humans and has caused mortality [10]. It is now known that nalidixic acid—the prototypic quinolone—is a good predictor for the reduced susceptibility to fluoroquinolones in salmonellae [16]. The increase in nalidixic acid resistance, in some way, may reflect the emergence of fluoroquinolone resistance.

Another worrisome situation is the observation in Taiwan of the upsurge of S. enterica Choleraesuis strains, a highly invasive non-typhoid Salmonella serotype, that are resistant to many antimicrobial agents [17]. Although the prevalence of S. Choleraesuis has increased to constitute ~10% of total non-typhoid Salmonella isolates in Taiwan, the rate of resistance to ≥1 antimicrobial agent has dramatically increased to as high as 98% of S. Choleraesuis isolates (table 1). Of the drug-resistant S. Choleraesuis isolates, ~90% are resistant to ampicillin, chloramphenicol, or sulfamethoxazole-trimethoprim, and ~70% are resistant to ciprofloxacin (MIC, ≥4 μg/mL) [18]. Aggravating this situation was the appearance of a hitherto-unknown S. Choleraesuis clinical isolate simultaneously resistant to ceftriaxone and ciprofloxacin in 2002 [19]. The emergence of such a resistance trait in S. Choleraesuis poses a serious threat to human health and should be monitored closely.

**MECHANISMS OF FLUOROQUINOLONE AND EXTENDED-SPECTRUM CEPHALOSPORIN RESISTANCE**

Conventional antibiotics, such as ampicillin, chloramphenicol, and sulfamethoxazole-trimethoprim, are no longer an appropriate choice for the treatment of invasive salmonellosis. Fluoroquinolones and extended-spectrum cephalosporins were 2 types of agents that had remained relatively effective and, thus, would have been a proper choice under such circumstances. Depressingly, however, as described above, there has been worldwide emergence of resistance to these agents. Thus, the drug resistance situation puts a physician in a quandary regarding the choice of proper antimicrobial agents for the treatment of patients infected with resistant salmonellae. Accordingly, it would be advantageous for a clinician to have clear background information on the drug resistance situation. The mechanism of resistance to these agents is summarized and discussed below.

**Fluoroquinolones.** Like other members of the Enterobacteriaceae, resistance to fluoroquinolones in salmonellae is mostly due to mutations in the quinolone resistance–determining region (QRDR) of the DNA gyrase genes [20]. The most frequently observed point mutations in gyrA of fluoroquinolone-resistant salmonellae are the changes of the amino acids at codon 83 from serine to phenylalanine, tyrosine, or alanine and at codon 87 from aspartic acid to glycine, asparagine, or tyrosine [20]. The amino acid changes associated with mutations in gyrB, such as the substitution of tyrosine for serine at codon 463, also contribute to the resistance of Salmonella serotypes to fluoroquinolones [20]. Mutations in parC, which caused amino acid changes at codon 80 from serine to isoleucine or arginine, have recently been reported in S. Typhimurium and S. Choleraesuis [19, 21, 22].

Another mechanism that may participate in fluoroquinolone resistance in salmonellae is the recently reported AcrAB-ToLC efflux system and its regulatory genes, mcrARAB and soxRS [20]. Both target gene mutation and efflux pumping are important mechanisms in triggering high-level resistance to fluoroquinolones (MIC, ≥32 μg/mL). However, the active efflux system seems to play a major role: when the acrB gene is inactivated, the resistance level to fluoroquinolones is significantly reduced, even when multiple mutations in target genes are present [21]. A recently identified efflux pump inhibitor, Phe-Arg-naphthylamide, was shown to display efficient inhibitory effect and thus was considered as a potential therapeutic agent in combination with fluoroquinolones to treat infections caused by Salmonella serotypes with high-level resistance to fluoroquinolones [21]. The actual effect of such efflux pump inhibitors, however, needs further clinical evaluation.

Alterations in the expression of outer membrane proteins (such as OmpF) or lipopolysaccharides have been discovered.
Table 1. Global status of antimicrobial resistance in nontyphoid *Salmonella* isolates recovered from human sources in the past decade.

<table>
<thead>
<tr>
<th>Country</th>
<th>Year(s) of study</th>
<th>Salmonella enterica serotype</th>
<th>No. of isolates</th>
<th>Any antibiotics</th>
<th>AM</th>
<th>C</th>
<th>SXT</th>
<th>CIP</th>
<th>CRO/CTX</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>1989–1990</td>
<td>Unspecified</td>
<td>758</td>
<td>31</td>
<td>13</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>[1]</td>
</tr>
<tr>
<td>Greece</td>
<td>1993</td>
<td><em>S</em>. Enteritidis</td>
<td>58</td>
<td>NA</td>
<td>25</td>
<td>24</td>
<td>4</td>
<td>0.3</td>
<td>NA</td>
<td>[2]</td>
</tr>
<tr>
<td>Thailand</td>
<td>1995</td>
<td>Unspecified</td>
<td>349</td>
<td>NA</td>
<td>20</td>
<td>17</td>
<td>8</td>
<td>0</td>
<td>5</td>
<td>[3]</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>1996</td>
<td><em>S</em>. Typhimurium</td>
<td>5849</td>
<td>90</td>
<td>80</td>
<td>75</td>
<td>86</td>
<td>12</td>
<td>NA</td>
<td>[5]</td>
</tr>
<tr>
<td>United States</td>
<td>1997</td>
<td><em>S</em>. Enteritidis</td>
<td>18,968</td>
<td>8</td>
<td>5</td>
<td>1</td>
<td>&lt;1</td>
<td>1</td>
<td>NA</td>
<td>[6]</td>
</tr>
<tr>
<td>France</td>
<td>1997</td>
<td><em>S</em>. Typhimurium</td>
<td>992</td>
<td>NA</td>
<td>73</td>
<td>56</td>
<td>9</td>
<td>NA</td>
<td>NA</td>
<td>[7]</td>
</tr>
<tr>
<td>Italy</td>
<td>1990–1998</td>
<td><em>S</em>. Enteritidis</td>
<td>1624</td>
<td>2.3</td>
<td>0.8</td>
<td>0.2</td>
<td>0.8</td>
<td>NA</td>
<td>0.4</td>
<td>[8]</td>
</tr>
<tr>
<td>Spain</td>
<td>2001</td>
<td>Unspecified</td>
<td>1051</td>
<td>73</td>
<td>45</td>
<td>26</td>
<td>14</td>
<td>0.6</td>
<td>0.2</td>
<td>[9]</td>
</tr>
<tr>
<td>Spain</td>
<td>2001</td>
<td><em>S</em>. Typhimurium</td>
<td>284</td>
<td>NA</td>
<td>80</td>
<td>73</td>
<td>19</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>2001</td>
<td><em>S</em>. Enteritidis</td>
<td>385</td>
<td>NA</td>
<td>23</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Taiwan</td>
<td>2003</td>
<td>Unspecified</td>
<td>675</td>
<td>69</td>
<td>44</td>
<td>49</td>
<td>31</td>
<td>8</td>
<td>1.5</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>S</em>. Choleraesuis</td>
<td>67</td>
<td>98</td>
<td>91</td>
<td>91</td>
<td>88</td>
<td>69</td>
<td>1.5</td>
<td>Su LH, unpublished data</td>
</tr>
</tbody>
</table>

**NOTE.** AM, ampicillin; C, chloramphenicol; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; NA, data not available; SXT, sulfamethoxazole-trimethoprim.

in quinolone-resistant salmonellae [20]. However, much work remains to be done to elucidate the contribution of these alterations in the membrane components to the fluoroquinolone resistance in salmonellae.

**Extended-spectrum cephalosporins.** Resistance to broad-spectrum cephalosporins is mainly due to the organism’s production of extended-spectrum cephalosporinases. It has been reported that salmonellae produce a variety of such enzymes, the majority of which are extended-spectrum β-lactamases (ESBLs; particularly CTX-M types) and AmpC β-lactamases (particularly CMY-2 type) that can hydrolyze cephalosporins as well as cephamycins [19, 23–25]. Other types of extended-spectrum cephalosporinases, such as SHV, TEM, ACC, PER, OXA, and DHA, have also been reported in salmonellae [26, 27].

The genes encoding these extended-spectrum cephalosporinases are carried by conjugative plasmids, transposons, or integrons. These mobile genetic elements could spread horizontally between enteric organisms. Thus, not only can antimicrobial resistance in salmonellae emerge because of the selection pressure derived from inappropriate antimicrobial use in food animals, but drug-susceptible salmonellae can also become resistant via the in vivo acquisition of drug resistance plasmids from other enteric pathogens in the intestinal tract of patients [23].

The evidence obtained in genotyping and plasmid analysis indicates that both clonal dissemination and horizontal transfer of resistance genes, such as *bla*<sub>CMY-2</sub>, are contributing to the spread of the resistance determinant [24]. Of the 2, the plasmid transmission through conjugation appears to play a more important role, because identical *bla*<sub>CMY-2</sub> is found in *Salmonella* isolates with distinct chromosomal DNA patterns [25]. We recently found that the spread of *bla*<sub>CMY-2</sub> among *Salmonella* strains also could occur through the mobilization of nonconjugative plasmids that carry the resistance gene [19].

Also, resistance plasmids have been shown to recombine with serotype-specific virulence plasmids of *Salmonella* and to form a virulence/resistance hybrid plasmid [28]. It appears that such evolutionary events endow salmonellae with not only the advantage to survive in an unfavorable drug environment but also an opportunity to disseminate into a new genetic lineage [29, 30], resulting in the overall increase in the prevalence of drug-resistant *Salmonella* infection in both humans and animals [31].

**SPREAD OF ANTIMICROBIAL-RESISTANT STRAINS**

Antimicrobial resistance arises in several ways, including acquisition of resistance genes via horizontal gene transfer and selection of resistant variants in the population. In the case of *Salmonella*, the situation is more complicated, because the use of antibiotics for therapeutic or preventive purposes in veter-
inary medicine and as growth promoters in animal feed may promote the emergence of resistance, thereby presenting a potential risk to public health from zoonotic infections [32]. Although the route of transmission of antimicrobial-resistant salmonellae is complex, evidence obtained in many epidemiological and laboratory studies suggest that the primary source of antimicrobial-resistant Salmonella infection is foods of animal origin [33]. Recent surveys with molecular techniques provide firm evidence indicating that the use of antimicrobial agents in food animals contributes to the development of antimicrobial-resistant salmonellae that cause infections in humans [12, 13, 32, 33]. In Taiwan, a survey by the National Health Research Institute indicated that 5 antibiotics critical to human medicine, including a fluoroquinolone (enrofloxacin), have been widely used in farms and feed mills [34]. Enrofloxacin can select Salmonella mutants resistant to nalidixic acid and a broad range of fluoroquinolones, including ciprofloxacin [35]. Two subsequent studies from Taiwan confirmed that the same genotypes were shared by both human and animal isolates of ciprofloxacin-resistant Salmonella [18, 22]. In Denmark, food animal industries relinquished the use of all antimicrobial growth promoters by 1999 on the basis of the expectation that removal of the selective antimicrobial pressure in animals would reduce the exposure of humans, through food, to antimicrobial-resistant bacteria from animals. Subsequent reports demonstrated that both antimicrobial resistance and the pathogen load of foodborne bacteria, including salmonellae, decreased significantly in food animals after the ban [36, 37]. The Danish experience is clearly showing that it is important and also possible to reduce resistance in human pathogens by curbing antibiotic use in food animals.

**CLINICAL IMPACT OF ANTIMICROBIAL RESISTANCE**

The influence of antimicrobial resistance in Salmonella species is quite extensive, reaching many areas. Acquisition of resistance genes adds complexity to laboratory diagnosis and complicates therapeutic outcomes. Antimicrobial resistance also affects the therapeutic regimen, leading to considerable public health concerns and substantial economic burden. Some means to cope with antimicrobial resistance are described below.

**Laboratory detection of resistance.** Although there are increasing reports of salmonellae producing extended-spectrum cephalosporinases, the guidelines suggested by the NCCLS for the screening of ESBL-production are still applicable only to Escherichia coli, Klebsiella pneumoniae, and Klebsiella oxytoca. Consequently, many clinical microbiological laboratories do not include Salmonella species in their routine ESBL screening. Failure to identify drug-resistant salmonellae may affect the choice of appropriate alternative antibiotics in the treatment of patients with invasive salmonellosis [23].

Similar problems occur in fluoroquinolone resistance. It is well known that high-level fluoroquinolone resistance (MIC, ≥32 μg/mL) is a cumulative result of stepwise mutations [20]. Reports have indicated that mutations in the QRDRs of the DNA gyrase genes can be found in Salmonella isolates expressing reduced susceptibility to fluoroquinolones, although according to the current NCCLS criteria, the MIC of ciprofloxacin still falls in the susceptible category [10]. The therapeutic effect of fluoroquinolone treatment on extraintestinal infections caused by these salmonellae is relatively poor [10]. Therefore, it was suggested that the current NCCLS fluoroquinolone breakpoints for the Enterobacteriaceae should be revised to adequately reflect the actual resistance status in Salmonella species [15]. Moreover, detection of resistance to nalidixic acid by disk diffusion is a sensitive and specific method for the screening of Salmonella isolates with reduced susceptibility to fluoroquinolones [16]. NCCLS therefore recommended in the 2003 guidelines that nalidixic acid may be used to detect such reduced susceptibility to fluoroquinolones in isolates recovered from patients with extraintestinal Salmonella infections [38].

**Therapy.** Patients with invasive salmonellosis require antimicrobial treatment. Increasing antimicrobial resistance may add to the difficulty or delay in administration of microbiologically effective therapy, leading to increased morbidity and mortality [39]. On the other hand, antimicrobial use causes a transient decrease in an individual’s resistance to colonization with noncommensal bacteria and increases the likelihood of infection on exposure to a foodborne pathogen, such as Salmonella species [40]. The additional “selective effect” of antimicrobial resistance results in an increase in vulnerability to infection with a drug-resistant pathogen among individuals receiving antimicrobial therapy for unrelated reasons [40]. It was estimated that, in the United States, antimicrobial resistance in nontyphoid Salmonella serotypes has resulted in an extra 29,379 infections annually, causing an additional 342 hospitalizations (or 8677 days of hospitalization) and 12 deaths [40].

In addition to the ill consequence of compromised antimicrobial treatment for invasive salmonellosis, resistant salmonellae may also complicate the treatment of other infections. In view of the increasing rate of antimicrobial resistance among common pathogens, including salmonellae, broad-spectrum agents are now more likely to be used for empirical therapy. For example, imipenem or cefepime might be used more frequently in a clinical setting where isolates that produced extended-spectrum cephalosporinases were frequently encountered. These antibiotics are usually more expensive and toxic, and more harmful to the commensurate microflora. Thus, a vicious cycle forms as the situation deteriorates, leading to great socioeconomic losses from the perspective of the patient, the hospital, and the whole society [41]. A feared fact is that the effectiveness of these drugs has been challenged by the recent
isolation of a *Salmonella* strain that was resistant to imipenem [42].

**CONCLUSIONS**

Widespread drug-resistant nontyphoidal salmonellae and the associated complications in the treatment of infection have constituted a serious threat to the public health. Treatment with conventional antibiotics, such as ampicillin, chloramphenicol, and sulfamethoxazole-trimethoprim, is now handicapped, and the increasing resistance to newer antimicrobial agents, such as fluoroquinolones and extended-spectrum cephalosporins, further aggravates the problem. One of the means of overcoming the resistance problem in salmonellae is to discontinue the overuse and misuse of antimicrobial agents in food animals [18, 32, 33, 36, 37]. Furthermore, because the resistance displayed by *Salmonella* serotypes reflects the environment in which the organism thrives, immediate action, including rigorous restriction of the use of extended-spectrum cephalosporins and fluoroquinolones in food animals and humans and reinforcement of infection-control measures in clinical settings, should also be taken. Continued surveillance for resistance and the inclusion of appropriate screening tests for extended-spectrum cephalosporinases in routine susceptibility testing for salmonellae are necessary.

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**References**


