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Secular trends in incidence and antimicrobial resistance among clinical isolates of salmonella at a university hospital in Taiwan, 1983–1999

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SUMMARY

The incidence and antimicrobial resistance among clinical isolates of salmonella at a university hospital in Taiwan between 1983 and 1999 are summarized in this report. A total of 7986 isolates were analysed. Serogroup B has been the most prevalent over the years, with an apparently continuous decline after 1995. Concordant decrease was also found among *S. choleraesuis* and *S. typhi* isolates in recent years. In contrast, the proportion of serogroup D strains increased significantly after 1996. *S. typhi* remained relatively susceptible to most of the antimicrobial agents examined. For non-typhoidal isolates, antimicrobial resistance to ampicillin (62%), chloramphenicol (67%), and sulfamethoxazole-trimethoprim (37%) was relatively higher than that reported elsewhere. Newer generation cephalosporins and fluoroquinolones remained effective over the years, although emerging resistance to these drugs has been noticed since 1992. A more prudent selection and use of antimicrobial agents, in both humans and animals, and a continuous surveillance of resistance are essential in the future.

INTRODUCTION

Salmonella, a genus belonging to the family *Enterobacteriaceae*, comprises a large and complex group of human pathogens that have long been associated with a wide spectrum of infectious diseases, including typhoid fever and non-typhoidal salmonellosis. Human salmonellosis is usually self-limiting and antimicrobial treatment is seldom required [1]. Early reports have indicated that antimicrobial treatment for uncomplicated gastroenteritis does not reduce the duration or severity of symptoms; in contrast, it may prolong faecal excretion at convalescence and result in the emergence of resistant strains [2]. Nevertheless, if the spread beyond the intestine occurs, effective antimicrobial treatment is essential, and the knowledge of the likelihood of resistance to commonly available drugs could be of considerable value to the clinicians.

Salmonellosis, non-typhoidal in particular, has been rampant in Taiwan [3–5]. With the increasing tourism from other countries in recent years and, since 1994, the mass introduction of foreign labour workers from Southeastern Asia, (amount to 1·3% of current local population), the epidemiology of human salmonellosis in this isolated island country may have been changing substantially. On the other hand, with the increasing use of extended-spectrum cephalosporins and fluoroquinolones, antimicrobial resistance may have further complicated this problem. This study was therefore conducted aiming to provide the clinicians a longitudinal view of the changing epidemiology and also the most recent information concerning salmonella.

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METHODS

Source of isolates

Records of salmonella clinical isolates by the Division of Microbiology, Department of Clinical Pathology in Chang Gung Memorial Hospital (CGMH) between 1983 and 1999 were reviewed. The CGMH is a 4000-bed university-affiliated medical centre located in northern Taiwan. The patients were virtually from the whole Taiwan district, including the main and scattered islands.

Microbiological studies

All isolates were cultured and identified according to standard methods [6]. No major changes in the policy concerning identification of salmonella were made through the years. Salmonella isolates were checked with O antisera (Difco) for their serogroups by the slide agglutination method. The antimicrobial susceptibility of these isolates was investigated by the standard disk-diffusion method [7] for non-blood isolates and by the microbroth dilution method [8] for blood isolates. The antimicrobial agents examined included ampicillin (10 μg), cefepime (30 μg), cefixime (5 μg), ceftazidime (30 μg), ceftizoxime (30 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), and sulfamethoxazole/trimethoprim (SXT) (1·25/23·75 μg). Ceftazidime and ceftriaxone were not available until 1991, while ciprofloxacin and ceftizoxime became available only after 1996 and 1997, respectively. Cefixime and cefepime had been introduced into this hospital since 1998. Susceptible and resistant isolates were defined according to the criteria suggested by the National Committee for Clinical Laboratory Standards [7, 8]. The isolates in the 'intermediate' category were deemed as 'resistant' in this study.

Statistical analysis

The χ^2 test was used to determine the significance of differences. A difference was considered statistically significant with a P value less than 0·05.

RESULTS

Trends in annual isolate numbers

A total of 7986 salmonella isolates were analysed in this study. The number of annual isolates increased from 94 in 1983 to 774 in 1999 (average 470; maximum 910 in 1995). Compared to the total bacteria isolated in this laboratory (16851 in 1983 and 52920 in 1999), the proportion of salmonella isolates significantly increased ($P < 0·0001$) from 0·56% in 1983 to 1·46% in 1999 (average 1·39%; maximum 2·06% in 1995). For all salmonella isolates, a significant increase was observed among non-blood isolates (34 in 1983 and 650 in 1999) compared to blood isolates (60 in 1983 and 124 in 1999; $P < 0·0001$).

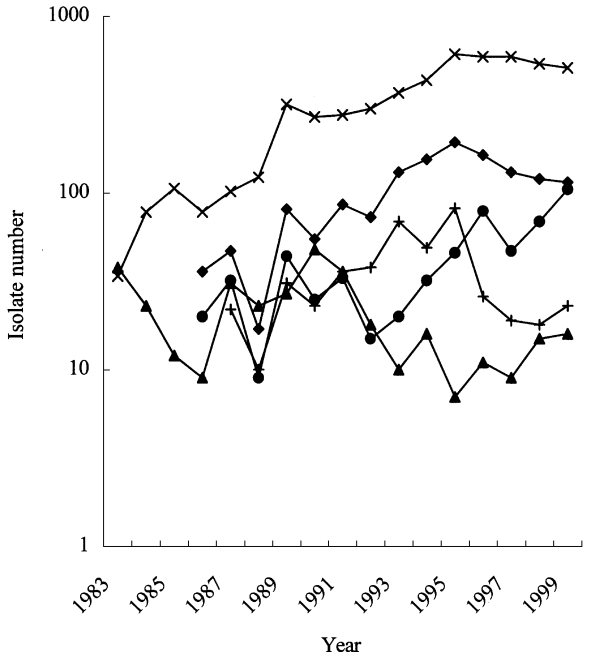

Fig. 1. Secular trends in the annual isolate numbers for serogroups B (×), C (◆), D (●), *S. choleraesuis* (+), and *S. typhi* (▲) in CGMH, Taiwan, 1983–99.

The numbers of annual isolates for major serogroups of salmonella are shown in Figure 1. Serogroup B has been the most prevalent among all salmonella isolates through the years. The number of annual isolates increased from an average of 87 in 1983–8, 328 in 1989–94, to 569 in 1995–9, while a slight yet continuous decrease was observed after 1995. The proportion of serogroup B to the total salmonella isolates remained stationary around 60–70% (average 66·8%) in these years.

Serogroup C represented another large group isolated, second only to serogroup B (Fig. 1). The number of annual isolates increased steadily to a maximum in 1995 and declined thereafter. The proportion of serogroup C to the total salmonella isolates fluctuated around 13–22% (average 16·7%). *S. choleraesuis* is one of the major serovars within serogroup C, constituting an average of 31·7% of the

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isolates in the group. However, the proportion of *S. choleraesuis* among the serogroup C isolates dropped significantly from 41·1% before 1995 to 16·2% after 1996 ($P < 0{\cdot}0005$). Compared to the overall salmonella isolates, which averaged 367 before 1995 and 803 after 1996, the figures amounted to a significant decrease in the number of annual isolates from an average of 28 before 1995 to 22 after 1996 ($P < 0{\cdot}0005$).

Serogroup D formed the third large group among all salmonella isolates, consisting of 7·2% of the whole population (Fig. 1). The number of annual isolates was around 21 before 1995. After 1996, a significant increase to 75 was noted ($P < 0{\cdot}05$).

S. typhi constituted 4·2% of the total salmonella population with an average annual isolate number of 20 (Fig. 1). However, a significant decrease was observed for this figure from 26 before 1991 to 13 after 1992 compared to the whole salmonella isolate numbers (248 before 1991 and 719 after 1992; $P < 0{\cdot}0001$). As to *S. paratyphi*, there were only 0–4 isolates annually, which was less than 1% of all salmonella isolates.

Serogroup E was a small group consisting of 12 isolates or 2·6% of the whole salmonella population annually. No significant change in the number of isolates was found through the years.

Invasion index

The invasion index, which is defined as the number of blood isolates per 100 total isolates, for some major serogroups is shown in Figure 2. The most invasive strains were *S. typhi* and *S. choleraesuis*, which indexed at 66 and 64, respectively. In comparison, isolates in serogroup C other than *S. choleraesuis* showed a much lower invasion index at 15. The invasion index for serogroup D varied between 20 and 65, with an average of 36. Serogroup B isolates showed the most constant invasion index at 17.

Trends in antimicrobial susceptibilities

Figure 3 shows the trends in antimicrobial resistance to ampicillin, chloramphenicol, and SXT for some major salmonella serogroups over the years. For serogroup B (Fig. 3A), the resistance to ampicillin and chloramphenicol increased concordantly from 50% to 80% after 1988 ($P < 0{\cdot}001$). In comparison, the resistance to SXT appeared to be lower, with a trough

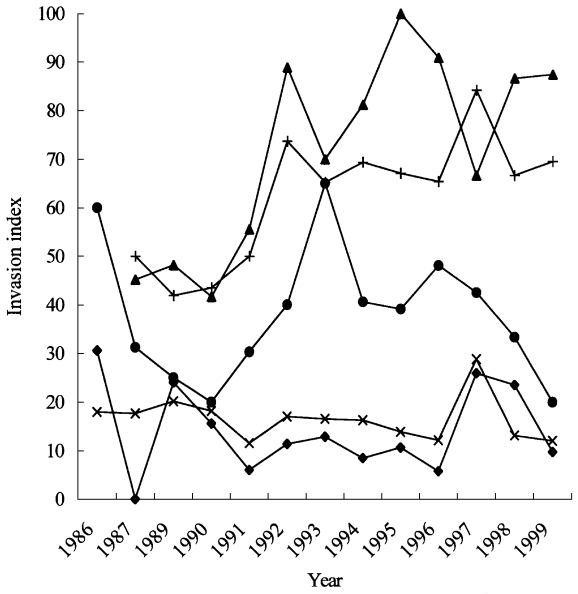

Fig. 2. The invasion index, or the number of blood isolates per 100 total isolates, for serogroups B (×), C (excluding *S. choleraesuis* (◆), D (●), *S. choleraesuis* (+), and *S. typhi* (▲) in CGMH, Taiwan, 1986–99.

of 25% in 1987 followed by a slight but steady increase to 44% in 1999.

For serogroup C, reliable data were not available until 1991. The results are shown in Figure 3B for those isolates excluding *S. choleraesuis*, and Figure 3C for *S. choleraesuis*. In general, *S. choleraesuis* isolates appeared to be more resistant to ampicillin, chloramphenicol, and SXT than the other serogroup C isolates. A similar trend was observed in the resistance to both ampicillin and SXT among non-Choleraesuis serogroup C isolates. However, the rate of SXT resistance increased to 57–58%, a figure similar to that of *S. choleraesuis* isolates, after 1995. With regards to chloramphenicol resistance, no significant change was found over the years among non-Choleraesuis serogroup C isolates, while a significant decline from 86% to 68% ($P < 0{\cdot}05$) was observed after 1996 for *S. choleraesuis* isolates.

Similar trends in the antimicrobial resistance to ampicillin, chloramphenicol, and SXT were observed for serogroup D isolates, especially after 1992 (Fig. 3D). The resistance increased to 60–70% in 1995, followed by a decrease to 10–20% in recent years.

S. typhi isolates were highly susceptible to almost all the antibiotics analysed, except that a resistance of approximately 10% to ampicillin and chloramphenicol was observed in recent years.

The antimicrobial resistance to the newer generation cephalosporins and ciprofloxacin is shown in

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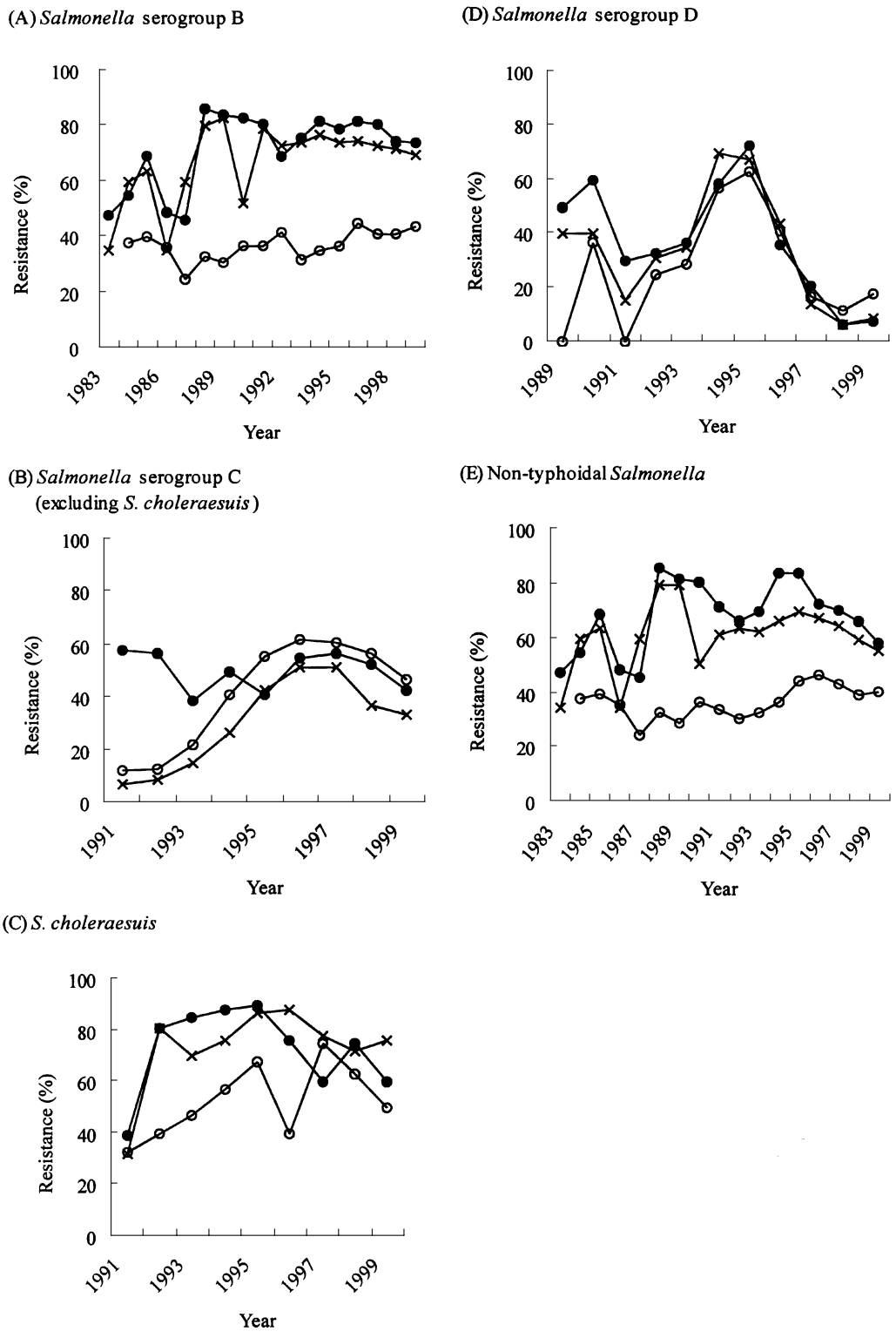

Fig. 3. Secular trends in the antimicrobial resistance to ampicillin (×), chloramphenicol (●), and sulfamethoxazole/trimethoprim (○) among (A) serogroup B, (B) serogroup C (excluding *S. choleraesuis*), (C) *S. choleraesuis*, (D) serogroup D, and (E) non-typhoidal salmonella isolates in CGMH, Taiwan.

Table 1. All *S. typhi* and *S. paratyphi* isolates tested remained susceptible to these agents. For non-typhoidal salmonella, the resistance rates to these antibiotics were generally under 2·5%. A higher resistance to such agents was found among serogroup E isolates, and all the resistant strains were isolated in

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Table 1. *Antimicrobial resistance to several newer generation cephalosporins and ciprofloxacin among salmonella isolates in CGMH, Taiwan, 1991–9*

	Antimicrobial agent* (number of isolates tested/% of resistance)					
Salmonella	CFM	CAZ	ZOX	CRO	FEP	CIP
S. typhi	1/0·0	130/0·0	38/0·0	94/0·0	20/0·0	66/0·0
S. paratyphi	1/0·0	8/0·0	5/0·0	3/0·0	4/0·0	3/0·0
Non-typhoidal	1047/1·9	2829/0·6	1934/1·2	3649/0·7	231/0·0	2303/2·2
Serogroup B	751/1·6	1825/0·8	1362/1·4	2439/0·7	141/0·0	1611/2·9
Serogroup C†	143/0·0	356/0·6	264/0·4	499/0·4	17/0·0	349/1·1
Serogroup D	116/2·6	243/0·0	199/0·0	312/0·3	42/0·0	231/0·0
Serogroup E	22/22·7	82/0·0	35/8·6	105/4·8	0/0·0	43/2·3
S. choleraesuis	7/0·0	290/0·0	54/0·0	256/0·0	29/0·0	45/0·0
Others	8/0·0	33/0·0	20/0·0	38/0·0	2/0·0	24/0·0
Total	1049/1·9	2967/0·6	1977/1·2	3746/0·7	255/0·0	2372/2·2

* CFM, cefixime; CAZ, ceftazidime; ZOX, ceftizoxime; CRO, ceftriaxone; FEP, cefepime; CIP, ciprofloxacin.
† Excluding *S. choleraesuis*.

1999. Various resistance rates/isolate numbers were observed among the other serogroups. The emergence of resistance could be traced back to as early as 1992, in which year one isolate resistant to ceftazidime and four to ceftriaxone among serogroup B were noted. As to the only fluoroquinolone studied in this report, ciprofloxacin, resistance had been found among serogroup B isolates since the antibiotic was first introduced in this hospital in 1996. The resistant rates varied between 2 and 5% during the past 4 years.

In vitro activity of conventional antibiotics against non-typhoidal salmonella

Figure 3E summarizes the antimicrobial resistance to ampicillin, chloramphenicol, and SXT for all non-typhoidal salmonella isolates, including serogroups B, C, D and E. The average resistance to SXT was 37%, which was significantly lower than that to ampicillin (62%) and chloramphenicol (67%) ($P < 0{\cdot}0001$). However, the resistance appeared to increase gradually after a trough of 25% in 1987. More fluctuation was observed for the trends in the resistance to ampicillin and chloramphenicol compared to that to SXT.

When different serogroups were compared, the resistance to SXT varied among the non-typhoidal serogroups, with the highest rate being 53% for *S. choleraesuis* and the lowest 27% for serogroup D ($P < 0{\cdot}005$). Significant difference was also observed when the resistance to ampicillin was compared between non-Choleraesuis serogroup C isolates (30%) and *S. choleraesuis* (72%; $P < 0{\cdot}0001$). As to the resistance to chloramphenicol, there was a significant difference between serogroup B and serogroup D isolates (average resistance rate, 72% *vs.* 38%, $P < 0{\cdot}0005$).

DISCUSSION

This report covers the longest surveillance period for the longitudinal perspective in incidence and antimicrobial resistance among salmonella clinical isolates in the literature. Data reported in this study were generally similar to those of earlier studies from Taiwan in terms of distributions among various serogroups and antimicrobial resistance to the conventional antibiotics. This may be due to the fact that salmonella causing human illness were clonal in origin, and there may be successive clones circulating island-wide, as has been reported elsewhere [9]. Moreover, this report further highlights current situations regarding the increasing resistance to those traditional antibiotics and the emerging resistance to the newer drugs in recent years.

Similar problems have been found in other countries, where the emerging resistance to ciprofloxacin [10–13] and extended-spectrum cephalosporins [14] has been of particular concern. Since these reports were generally accumulated before 1997 with fewer isolate numbers studied, our data from a larger population further confirm the profoundness of these problems.

As to those conventional antimicrobial agents, including ampicillin, chloramphenicol and sulfamethoxazole-trimethoprim, the resistance was relatively

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higher in Taiwan than in other countries [14–18], even those in the neighbouring areas [12, 19]. Although increasing antimicrobial resistance to ampicillin [17, 18], chloramphenicol [18], and sulfamethoxazole-trimethoprim [4, 12] was observed during most of the studying periods in this as well as earlier reports, a reverse trend has been observed in recent years [14, 16]. It was hypothesized that the decline in resistance could be due to the replacement of conventional antibiotics by new quinolones and third-generation cephalosporins in the treatment of salmonella infections and other bacterial diarrheas. This speculation might also explain similar findings in the present study that the decrease in antimicrobial resistance to the old agents was accompanied with the emergence of resistance to the third-generation cephalosporins since 1996.

In contrast to the prevalence of multidrug-resistant *S. typhi* observed in other countries [20, 21], high susceptibility to either new or old antimicrobial agents was demonstrated for this organism in the present study. However, resistance to ampicillin and chloramphenicol was occasionally observed in recent years. This resistance was also reflected in a higher MIC_{90} for chloramphenicol in a previous report from this hospital [22]. The selection of antibiotics should be more judicious in the treatment of typhoid fever before the antimicrobial resistance emerged to be a problem for *S. typhi* in Taiwan.

The increase in the number of annual isolates may further worsen the problem of antimicrobial resistance among salmonella in Taiwan. The findings that serogroup B, most of which being *S. typhimurium* [5, 23], is the commonest among the whole salmonella population [14, 24, 25], the significant increase in the isolation of serogroup D salmonella in recent years [15, 24–26], and the concomitant decline of *S. typhi* [24] and serogroup B isolates [25, 26] were similar to situations reported from the other parts of the world.

Khakhria et al. [25] attributed their observation of the decline in the isolation rate of *S. typhimurium* to the lower consumption of beef and pork and increased consumption of poultry. In Taiwan, there was an epidemic of swine mouth-foot disease in 1997. A large-scale pig massacre was performed in an attempt to terminate the outbreak. A study conducted afterward has proved that the incidence of bacteraemia associated with *S. choleraesuis* reduced significantly after the pig massacre [27]. These incidences support our present finding that both *S. choleraesuis* and serogroup B isolates decreased concordantly in recent years. On the other hand, *S. enteritidis*, a major serovar among serogroup D isolates in Taiwan (Su, unpublished data), has been shown to be closely related to layer and broiler flocks [28, 29]. As a consequence of the swine mouth-foot disease, the increasing consumption of poultry may contribute to the increase in the serogroup D isolates observed in recent years. A recent study has found that approximately 88% of broiler flocks and 49% of broilers in Taiwan were contaminated with salmonella [30]. A more detailed surveillance of a larger scale to study the distribution of salmonella serotypes among food animals may be able to provide a clearer aspect regarding the salmonella infections in Taiwan.

In conclusion, the annual isolate numbers of salmonella have been increasing in Taiwan. The resistance to conventional antibiotics is relatively higher than those reported from other countries. With the introduction of newer antimicrobial agents, the emerging resistance to these agents has apparently become an important issue that needs more attention. A more cautious selection and use of antimicrobial agents, in both humans and animals, along with a continuous monitor of resistance are critical to combat the increasing antimicrobial resistance in salmonella.

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