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Review Article

Bacteremia caused by *Salmonella enterica* serotype Choleraesuis in Taiwan

Shio-Shin Jean[1], Jen-Yu Wang[2], Po-Ren Hsueh[3]

[1]Departments of Internal Medicine and Intensive Care Medicine, Min-Sheng General Hospital, Taoyuan; [2]Department of Internal Medicine, Cardinal Tien Hospital, Fu Jen Catholic University College of Medicine, Taipei; and [3]Departments of Laboratory Medicine and Internal Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan

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Since 1995, there has been a steady increase in the number of reported cases of *Salmonella enterica* serotype Choleraesuis (*S.* Choleraesuis) sepsis in Taiwan. Representative Taiwanese survey data from 1996 to 2004 revealed that these adult patients with *S.* Choleraesuis bacteremia presented with primary bacteremia (57%, especially immunocompromised hosts), mycotic aneurysm (16%), and fever (86%) predominantly. *S.* Choleraesuis septicemia demonstrated a higher invasion index (with secondary involved sites) than other *Salmonella* spp. In swine experiments, the inoculation dose of 10^3 colony forming units *S.* Choleraesuis was cleared without apparent sequelae. Transmission of specific strains (with mutations of *GyrA* and *parC*, subsequently resistance to fluoroquinolones) from swine, and the acquisition of genes (CMY-2, *AmpC* complex) encoding beta-lactamases (with resistance to extended-spectrum cephalosporins) have been implicated in the evolution of multiresistant phenotypes of *S.* Choleraesuis. The virulence plasmid of *S.* Choleraesuis (pSCV), and other genes mediating adhesion to the epithelial cell membrane of the gastrointestinal tract, were considered important pathogenic factors for *S.* Choleraesuis. Vaccines for domestic animals combined with effective controls on antibiotic use offer the greatest potential to control the increasing impact of *S.* Choleraesuis on humans.

Key words: Beta-lactamases, cephalosporins, fluoroquinolones, *Salmonella enterica*, vaccines

Introduction

Non-typhoidal salmonellosis (including *Salmonella enterica* serotype Typhimurium [*S.* Typhimurium] DT104, and *Salmonella enteritidis*) has persistently ranked among the major foodborne diseases worldwide [1]. Clinically, antimicrobial treatment is not indicated in most patients. Characteristically, *Salmonella enterica* serotype Choleraesuis (*S.* Choleraesuis), a highly host-adapted pathogen, usually causes swine paratyphoid. This organism is notorious for its extreme invasiveness and pathogenic nature in humans, frequently causing septicemic disorders with scarce involvement of the gastrointestinal tract (primary bacteremia). Recently, antimicrobial resistance not only to older (ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole) but also to newer drugs (extended-spectrum cephalosporins [ESCs], and fluoroquinolones since 2000) used in the treatment of *S.* Choleraesuis has become a major public health issue in Taiwan.

In 1995, Chan et al first reported a 10-case series of salmonellosis in patients with aortic mycotic aneurysms, which revealed the endemic nature of this infection in Taiwan. *S.* Choleraesuis accounted for 80% of *Salmonella* etiologies in their series [2]. Subsequently, this organism has accounted for increased case numbers of salmonellosis in Taiwan. The increasing importance of *S.* Choleraesuis infections has stimulated research efforts on aspects such as clinical spectrum, pathophysiology, epidemiology, ecology, genetics, and cellular structure. This review focuses on the clinical impact, mechanisms of antimicrobial resistance and genetics/pathogenesis of

Corresponding author: Po-Ren Hsueh, Departments of Laboratory Medicine and Internal Medicine, National Taiwan University Hospital, National Taiwan University College of Medicine, Taipei, Taiwan.
E-mail: hsporen@ha.mc.ntu.edu.tw

S. Choleraesuis infections. Associations with other non-typhoid *Salmonella* infections, including the spectrum of clinical manifestations, outcomes, trends of antibiotic resistance, molecular mechanisms of resistance, virulence, and antimicrobial therapy, are also analyzed.

Disease Prevalence

An important representative study evaluated 81 patients with *S.* Choleraesuis bacteremic episodes at the National Taiwan University Hospital (NTUH), a 2000-bed university-affiliated general hospital located in northern Taiwan, and having a patient population from all parts of Taiwan, from 1996 through 2004 (including case proposed by Sun et al [3]), and analyzed detailed clinical and microbiologic data [4]. The annual case number and incidence (cases per 100,000 discharges) of adult patients with *S.* Choleraesuis bacteremia during each year is illustrated in Fig. 1. Although the epidemic of foot-and-mouth disease in Taiwan swine in 1996 led to the slaughter of many pigs, the annual case number gradually rose after 1999. In the same time period, the other six Taiwanese patients (one in 1996, the other one in 2002, and the remaining four cases diagnosed in 2003) with *S.* Choleraesuis bacteremia were obtained via *MEDLINE* search [5-8]. The trend of gradually increased annual case numbers of this infection predicts its emergence in the future.

Clinical Features and Outcomes

From the results of a study by Wang et al [4], male patients dominated (63%) and all patients had various underlying illnesses (including immunosuppressive diseases, gastrointestinal disorders, cardiovascular diseases mainly encompassing hypertension and heart failure, chronic renal failure, and skeletal disorders). The percentage of patients with underlying illnesses was higher than that in a previous investigation of *S.* Choleraesuis sepsis in Taiwan by Chiu et al (78%) [9]. Similar to other previous surveys of non-typhoid salmonellosis in adults from Taiwan, certain underlying illnesses [10-13], previous administration of immunosuppressive medication (cytotoxic chemotherapy, steroid) [11] and acquisition of specific serotypes (Choleraesuis, Dublin, Heidelberg, Virchow, Schwarzengrund and Panama) [10-14] were predisposing factors in different series. With respect to different clinical presentations (i.e., primary vs secondary bacteremia) among respective age groups, Chiu et al advocated that primary bacteremia (without preceding gastroenteritis) predominated in adult population, and it was associated with specific serotypes (Enteritidis, Choleraesuis and Dublin) [15]. Among the cases reported by Wang et al, 57% of patients presented with primary septicemia [4]. The most common types of infection were mycotic aneurysm (16%), osteomyelitis (4%), wound infections (4%), enterocolitis (4%), urinary tract infection

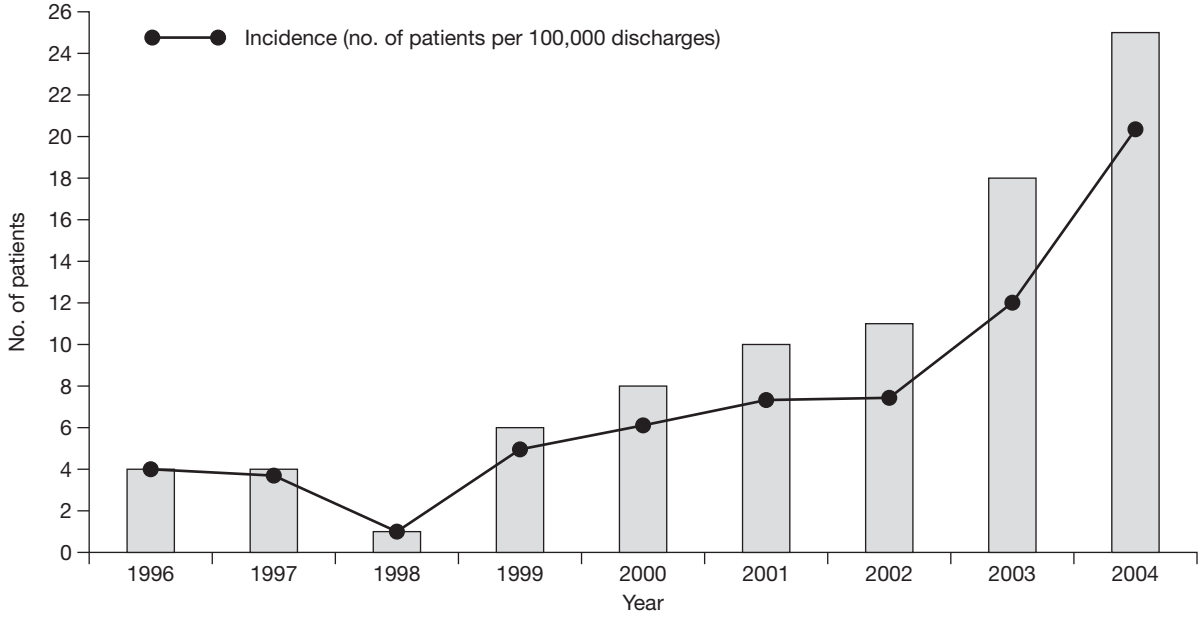

Fig. 1. Incidence and number of patients with *Salmonella* Choleraesuis bacteremia treated at National Taiwan University Hospital from 1996 to 2004.

(4%) and other miscellaneous localized infectious entities (9%). These data, consistent with a previous study from Taiwan [16], indicate that *S.* Choleraesuis usually has a high invasion index (the number of extraintestinal isolates divided by the total number of isolates). In contrast, another survey of small-case-number series in Taiwan noted that 21% (4/19) of *S.* Choleraesuis bacteremic patients subsequently developed metastatic focal infections, such as septic arthritis, cutaneous abscess, spontaneous bacterial peritonitis, and pneumonia [17]. The age range (50 to 84 years) of *S.* Choleraesuis bacteremic patients diagnosed of mycotic aneurysm in Wang et al's series [4] was exactly the same as reported in a prior study of non-typhoid *Salmonella* bacteremia in Taiwan [18]. In Taiwan, *Salmonella* mycotic aneurysm continues to be more common than in other parts of the world, and most of these aneurysms are due to *S.* Choleraesuis [2,4,18].

Clinically, more than five-sixths of patients initially presented with fever (86%), and one-quarter of patients presented with shock. In Wang et al's series [4], despite a high rate of *Salmonella* bacteremia among patients with various immunocompromised conditions, mycotic aneurysm was rarely found in this patient group. Additionally, *S.* Choleraesuis bacteremic patients with mycotic aneurysm and recurrent septicemic episodes were more likely to have ciprofloxacin-susceptible isolates (rather than -resistant ones), and both reached a statistically significant difference (*p* value of 0.028 and 0.033, respectively). The invasiveness of these ciprofloxacin-susceptible strains was probably due to factors other than antimicrobial resistance. In this series, the overall case-fatality rate was approximately 10%.

Interestingly, inclusive of 6 additional Taiwanese cases documented in the literature [5-8], a total of 7 patients (8%, 7/87) in this series had recurrent sepsis episodes. Among these patients, 3 had mycotic aneurysms, 2 had osteomyelitis and the remaining 2 patients had primary bacteremic episodes. With the exception of one patient who presented with recurrent septicemia from debrided mycotic aneurysm, the other 4 patients (two had osteomyelitis and the other two mycotic aneurysms) had relapse of infections despite adequate antimicrobial therapy. The presence of tissue damage or alteration of tissue structures (such as atherosclerotic plaque or diseased bones/joints) probably increased the susceptibility of these patients to seeding via repeated bacteremic episodes. Conservative antimicrobial management alone is unlikely to eradicate deep-seated foci of infection.

S. Choleraesuis isolates with resistance to ciprofloxacin were recovered from 53 patients (61%) in this series. There was no significant difference in age, sex, underlying diseases, recent hospitalization, clinical manifestations, or outcomes between the ciprofloxacin-susceptible and -resistant groups. A rapid increase in the annual incidence of ciprofloxacin resistance was found, from 0% before 2000, to 25% in 2000, and 80% in 2004. Multivariate analysis revealed that underlying immunosuppressive condition was an independent risk factor of primary bacteremia.

Environmental Ecology and Transmission

S. Choleraesuis is the most frequently isolated serotype from swine. In Taiwan, dense pig-raising areas in rural farms (with poorer sanitation than in those located in metropolitan areas) were associated with the more rapid spread of resistant clones [19]. *S.* Choleraesuis exhibited prolonged survival (13 months) in the desiccated state in nature [20], compared with 12 weeks for *S.* Dublin at the soil level [21]).

Notably, by autopsies and regular fecal culture of pigs, Gray et al reported that an inoculation dose of 10^6 colony forming units (CFU) did not cause mortality in swine, but that mild and transient clinical signs of disease occurred within 3 days of exposure. By contrast, the dose of 10^3 CFU was cleared without clinical signs and no apparent shedding [22]. Only a small proportion of infected swine were carriers at the end of a 9-week observation period. Dose-dependent correlation was noted. Gray et al also reported that after being exposed to a 3.0 $\log_{10}$ CFU dose environment, approximately 88% of naive pigs had confirmed shedding of *S.* Choleraesuis on anal swabs or fecal cultures up to day 11 post-inoculation [22]. These results demonstrated that the majority of swine, exposed to pigs excreting 2-3 log units of *S.* Choleraesuis per gram of stool (a low inoculation dose), would only become short-term (about 8 weeks post-inoculation) *S.* Choleraesuis shedders [23]. This information is useful for the development of veterinary vaccines, and for determination of critical isolation/observation periods before vaccinated swine are slaughtered.

Antimicrobial Drug Resistance and Treatment Options

According to the data from the survey of *S.* Choleraesuis in NTUH [4], resistance to chloramphenicol, ampicillin

and trimethoprim-sulfamethoxazole was found in 92%, 88%, and 78% of all isolates, respectively. The results were similiar to those of other studies in Taiwan [9,17]. These resistant rates were significantly higher than those of overall endemic non-typhoidal isolates for these antibiotics [16]. Notably, more than 60% of *S.* Choleraesuis isolates were resistant to ciprofloxacin.

Multiple antibiotic resistance of *S.* Choleraesuis is a serious management problem often presenting with tenaciously metastatic infections in Taiwan. Similarly, Lee et al's retrospective study, covering the period from January 1998 through June 2000, found that about 60% of cases were infected with non-typhoid *Salmonella* strains exhibiting multidrug-resistant phenotype after precedent antibiotic administration [24]. Cohen and Tauxe proposed that precedent antimicrobial use was an important finding in salmonellosis associated with: (i) converting multidrug-resistant *Salmonella* colonization into symptomatic infections; and (ii) culminating in lowering of the infectious threshold dose to cause disease [25]. Data from 2 medical centers in Taiwan revealed a high (18-21%) mortality rate for patients with severe immunocompromised status (hematological malignancies, liver cirrhosis, systemic lupus erythematosus, chronic renal failure), demonstrating the virulence of *S.* Choleraesuis in these patients [9,17].

Originally, ESCs (ceftriaxone, cefotaxime) and fluoroquinolones were recommended for the management of infections by multiresistant *Salmonella* species. However, since 1992, the emergence of infections caused by many different *Salmonella* serotypes showing resistance to these antibiotics has been increasingly reported in many countries [26-32]. The development of ciprofloxacin resistance in *S.* Choleraesuis for both human and veterinary isolates has been observed since the year 2000 in Taiwan [9,33,34]. The rate of resistance to ciprofloxacin was reported to range from 7.5% to 59% [9,19,33]. Use of fluoroquinolones (enrofloxacin) either at subtherapeutic levels as veterinary medication, or as growth promoters in animal feed, may encourage the development of resistance, presenting a potential threat to public hygiene and health from zoonotic infections [33]. Molecular surveillance revealed that swine served as the major reservoir for ciprofloxacin-resistant *S.* Choleraesuis [33,34]. The emergence of resistance to ESCs in endemic clinical *S.* Choleraesuis isolates poses a significant threat to the population of Taiwan [5,6,35, 36]. Other *Salmonella* serotypes with ceftriaxone-resistant phenotype had been verified to acquire beta (β)-lactamase CMY-2 gene from domestic animals (cattle) in the USA in 2000 [33].

Although quinolone-resistant genes were identified in other bacteria (especially *Escherichia coli*), the evolution of distinctive mutations (for fluoroquinolone resistance) on chromosomes of *S.* Choleraesuis has been confirmed. All ciprofloxacin-resistant strains of *S.* Choleraesuis in Taiwan, which have emerged rapidly since 2000, harbor chromosomal mutations involving the substitution of phenylalanine for serine at position 83, and asparagine for aspartate at position 87 in *GyrA*. Mutation in *parC* resulting in an amino acid change from serine to isoleucine at position 80 was also documented in many ciprofloxacin-resistant *S.* Choleraesuis isolates from Taiwan [19,33,34,37]. Human and swine isolates were shown to have an identical pulsotype. Swine-to-human transmission of ciprofloxacin-resistant genes has been hypothesized. However, a Taiwan countrywide investigation of *S.* Choleraesuis strains, from both humans and pigs collected from 1997 to 2002, noted a significantly higher fluoroquinolone resistance rate among isolates from humans (22/30, 73%) than from swine (26/76, 34%). Whether multidrug-resistant *S.* Choleraesuis in humans originate in isolates derived from pigs remains debatable. Furthermore, these quinolone-resistant epidemic strains, resistant to nalidixic acid, were not necessarily resistant to three fluoroquinolones (ciprofloxacin, enrofloxacin and norfloxacin). This result indicates the probable development of much more complex resistance mechanisms to modified quinolones in addition to nalidixic acid [38].

With respect to endemic cephalosporinases in *Salmonella* spp., a study of *S.* Choleraesuis isolates collected from March 1999 to December 2002 in Taiwan found a ceftriaxone resistance rate of about 2% [9]. A more recent investigation by Yan et al, of 600 *Salmonella* isolates collected throughout Taiwan from January to May 2004, found overall ESC resistance rates of 3.3%, with 17.8% for *S.* Choleraesuis [39]. The development of various β-lactamases (hydrolyzing ESCs) in *Salmonella* strains may share certain features with resistance of *E. coli* to cefotaxime. The genes in *Salmonella* of either CTX-M, CMY-2 or *AmpC* complex, all encoding extended-spectrum β-lactamases, could be carried by conjugative plasmids, transposons or integrons. Chiu et al used shotgun cloning and *E. coli* transformants to demonstrate that the *AmpC* (bla_{CMY-2}) gene, which mediates ceftriaxone resistance in *S.* Choleraesuis, is located on a potentially transmissible

140-kb plasmid [36]. Surveys of antimicrobial resistance of *E. coli* and *Klebsiella pneumoniae* from 1997 to 2000 at a medical center in southern Taiwan, and investigation of plasmids extracted from ceftriaxone-resistant *Salmonella* strains for ribotyping (use of primers of bla_{CMY-2}, bla_{TEM-1} structural genes), have confirmed the presence of bla_{CMY-2}-containing plasmids with an identical restriction fragment pattern in *Salmonella* strains/*E. coli*/*K. pneumoniae* isolates. This finding is strongly suggestive of interspecies dissemination and horizontal transfer of the resistance determinants [36]. The transmission of bla_{CMY-2} between enteric organisms (predominantly *E. coli*, in the community milieu) in food animals (chicken, pork) and humans has been detected in both Taiwan [40] and in the USA (not clonal spread within a restricted region) [41]. In Taiwan, additionally, several ESBL (including CTX-M-3, SHV-12, and SHV-2a) enzymes were also documented in non-typhoid salmonellosis, which also showed ceftriaxone resistance [42]. Carbapenems have become the only effective antimicrobial agents for the management of invasive infections caused by these *S.* Choleraesuis strains exhibiting resistance to both ciprofloxacin and ESCs [5,6].

Drug efflux systems (including AcrB/MexB in *S.* Typhimurium, the tri-partite complex, targeting special substrates with lipophilic side chains) have been found to enable many Gram-negative bacteria to survive in adverse environments (containing β-lactams, other antibiotics, lipopolysaccharide, and heavy metals, etc.) [43]. Although the existence of drug efflux pumps has been identified in *S.* Typhimurium [44,45], further studies will be required to disclose whether a multidrug-efflux pump system is implicated in multiple drug resistance in *S.* Choleraesuis.

In addition to the impact of choice of empirical antimicrobial treatment on potentially multiresistant invasive salmonellosis, antimicrobial resistance in humans and animals has been linked to both virulence (illustrated by clinically higher hospitalization rates, prolonged illness and hospitalization) and genetic determinants of resistance [46,47]. In the past, a much higher case fatality rate (4.2%) was found in outbreaks caused by antimicrobial-resistant *Salmonella* (21 times that of patients with antimicrobial-sensitive isolates), especially in populations at extreme ages [48]. These results suggest that resistant isolates are more virulent than susceptible ones [37,46]. Chu et al demonstrated that *S.* Choleraesuis would become a multidrug-resistant strain by acquisition of *sul* II and bla_{TEM-1} genes, and sequentially by recombination of the virulence plasmid with the multiresistant components on the other plasmid [49]. These strains are expected to be associated with high mortality in human beings and many domestic animals, and are thus of particular concern in Taiwan.

Bacterial Genetics and Pathogenesis

In addition to the synthesis of cytotoxin (for promoting gastrointestinal epithelial damage and invasion) [50], *S.* Choleraesuis usually harbored a 50-kb virulence plasmid named pSCV (virulence plasmid of *S.* Choleraesuis), carrying the *spv* operon, but the size of this plasmid was reported to be in the range of 125 to 140 kb. Restriction fragment profile revealed that these heterogeneously-sized plasmids originated in the 50-kb plasmid [49]. All virulence plasmids were noted to contain two virulence factors, *spv* operon and *mig5* operon. The *spv* operon was noted to be required for complex invasion (spread beyond the initial site of infection, i.e., intestinal cells, through Peyer's patches, to mesenteric lymph nodes and the spleen after peroral inoculation) [51], enhancing the growth within cells of the reticuloendothelial system, thereby causing systemic illness in mice. The *spv* operon, consisting of four structural genes *spvABCD* and a positive regulatory gene *spvR* (within a highly conserved 8-kb region), is the critical marker of virulence of many *Salmonella* serotypes, and consequently more likely to result in bacteremia and multiorgan dissemination. The expression of these genes can be induced under many stresses (positively controlled by sigma factor *RpoS*), which is beneficial to its survival in the hostile environment [52]. The *mig5* gene is also important for bacterial colonization in the mouse spleen [37]. These characteristic virulence plasmids may further recombine with certain genes conferring antibiotic resistance, which impose a new dilemma regarding the logical choice of antimicrobial regimens.

Many other genes documented in most virulent plasmids of *Salmonella* spp. were determined, including the following: serum resistance genes *rsk* (resistance to bactericidal serum killing), *rck* (encoding an outer membrane protein, which confers resistance to complement killing), *traT* (responsible for encoding surface lipoprotein, and resistance of rough strains), and some minor fimbrial genes, such as *pef*, *faeH*, *faeI*, and *oriT*. In comparison with other virulence plasmids, *S.* Choleraesuis notably contains *traT* but lacks *rck* gene [37].

The genetic products associated with adhesion and colonization in the intestinal tract of hosts are also of

paramount importance in invasion and access to the epithelial cell membranes. The most well-studied gene is *pef* operon, a 7-kb region containing plasmid-encoded fimbriae (including the *pefBACD*, *orf5*, *orf6*, *pefI*, and regulatory/structural/assembly proteins of adherence fimbriae) [53]. Mediation of adhesion to the epithelia of alimentary tract by *pef* fimbriae leads to fluid accumulation in murine gut. These genes have a decisive role in host adaptation to *S.* Typhimurium.

Chiu et al recently demonstrated that the genome sequence of *S.* Choleraesuis has evolved to be more adapted (and more pathogenic) to humans (than *S.* Typhimurium, *S. typhi*) as shown by several observations: (i) mutations in the genes involving bacterial chemotaxis signal-transduction pathways (increasing smooth swimming of the bacteria, and allowing more effective interactions with host hyper-invasiveness); (ii) inactivation of *acrR* (the regulatory gene of AcrAB-TolC efflux system) led to AcrAB over-expression, increasing ciprofloxacin resistance, and suggesting the role of an efflux pumping mechanism; and (iii) the presence of protein, homologous to von Willebrand factor type A domain of humans, causes septic vascular thrombi (via collagenase-mediated vascular injury) [54].

Preventive Measures

In countries with a high ciprofloxacin resistance rate of *S.* Choleraesuis such as Taiwan, regular surveillance projects conducted by the Government are needed to monitor trends in: (i) veterinary antimicrobial use; and (ii) antibiotic resistance rates among veterinary bacterial isolates. Prohibition of the use of certain antibiotics as growth promoters should be seriously considered. In the future, improved industry standards for raising pigs and for slaughtering and processing of pork would be beneficial in decreasing the transmission of *S.* Choleraesuis to human beings.

Vaccination for farm animals, especially pigs, is considered the most logical method for lowering the potential for transmission of *S.* Choleraesuis and thus for preventing this drug-resistant pathogen entering the human food chain. In comparison with *aro* (aromatic compound derivatives) and *gal*E mutant vaccines [55], live attenuated vaccines, such as the derivative of delta*cya* delta*crp-cdt* double mutation (impairing the ability of synthesizing cAMP/its receptor protein) without deletion of the *pmi* gene (encoding phospho-mannose isomerase, responsible for synthesizing lipopolysaccharide in *S.* Choleraesuis) [56,57], was demonstrated to provide immunity in pigs, and was associated with better subsequent weight gain. However, none of these effective vaccines for swine have been introduced in Europe (except Germany) or other parts of the world [37]. Implementation of policies regarding their use in animals at a national level thus appears to remain inadequate in many countries.

Conclusions

S. Choleraesuis is associated with a higher mortality rate in humans than other *Salmonella* serotypes. Its clinical spectrum is diverse and protean, including predominant septicemia (primary), aortitis, and many other localized infections. The organism has been recently recognized worldwide, due to its tendency to occur in the increasing population of patients with underlying systemic diseases, particularly those involving immunocompromised disorders, and the elderly. The development of resistance to ampicillin/chloramphenicol/trimethoprim-sulfamethoxazole and more recently resistance to fluoroquinolones and ESCs by *S.* Choleraesuis, has made the choice of adequate empirical antimicrobial agents more difficult, and lead to more prolonged and severe clinical courses with higher mortality rates. Currently, isolates of fluoroquinolone-resistant *S.* Choleraesuis are considered to be transmitted through swine, and ESC-resistant *S.* Choleraesuis strains were found to acquire CMY-2-like genes from other enteric Gram-negative bacteria. Recently, multidrug efflux pump mechanisms have been noted to confer a resistant phenotype for other *Salmonella* serotypes. Policies for the judicious administration of antibiotics in both agricultural and veterinary fields may need more rigorous implementation in order to prevent the emergence of resistance. The implementation of rigorous vaccine programs for domestic animals, combined with effective policies to control antibiotic use to limit the emergence of resistant strains, may offer the greatest potential to control the increasing impact of this disease in humans.

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