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Current focus

Antimicrobial resistance of foodborne pathogens

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Abstract

Emergence of bacterial antimicrobial resistance has become a serious problem worldwide. While much of the resistance observed in human medicine is attributed to inappropriate use in humans, there is increasing evidence that antimicrobial use in animals selects for resistant foodborne pathogens that may be transmitted to humans as food contaminants.

Keywords: Antibiotic resistance; Food borne pathogens

1. Introduction

Foodborne illnesses result in a major public health impact in the US and around the world. According to recently published CDC (US Centers for Disease Control and Prevention) data, foodborne diseases account for approximately 76 million illnesses, 325,000 hospitalizations, and 5000 deaths each year in the US alone [1]. Five pathogens account for over 90% of estimated food-related deaths: *Salmonella* (31%), *Listeria* (28%), *Toxoplasma* (21%), Norwalk-like viruses (7%), *Campylobacter* (5%), and *Escherichia coli* O157:H7 (3%). Although many of these diseases result in a self-limiting diarrheal illness in humans, severe invasive disease or prolonged illness in immunocompromised individuals can occur and may require antimicrobial therapy. Development of antimicrobial-resistant foodborne bacterial pathogens can potentially compromise human drug treatments.

It is now collectively acknowledged that the use of antimicrobials in both animals and humans can select for resistant bacterial populations. In food animals, antimicrobials are used for the control and treatment of bacterial associated infectious diseases as well as for growth promotion purposes. An undesired consequence of antimicrobial use in animals is the potential development of antimicrobial-resistant zoonotic foodborne bacterial pathogens and subsequent transmission to humans as food contaminants. In fact, emerging antimicrobial resistance phenotypes have been recognized among multiple zoonotic pathogens including *Salmonella enterica* serovar Typhimurium (e.g. definitive phage type (DT) 104), *E. coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, and *Yersinia enterocolitica*. The remainder of this review will concentrate on the development and characterization of antimicrobial resistance within the above-mentioned foodborne bacterial pathogens.

2. Shiga-toxin-producing *E. coli*

Shiga-toxin (Stx)-producing *E. coli* (STEC) were first recognized as an emerging human pathogen in 1982 when *E. coli* O157:H7 was implicated in two outbreaks of hemorrhagic colitis associated with consumption of undercooked ground beef [2]. Since then, more than 200 STEC serotypes have been identified and isolated from animals, food and other sources. However, only about 60 of these STEC serotypes have been associated with human disease. Among the STEC, O157:H7 is the classical serotype that was first associated with enterohemorrhagic diseases in the early 1980s and has been the cause of large foodborne outbreaks in North America, Europe, and Japan [3]. Among the non-O157 STEC foodborne outbreaks, serotypes O26 and O111 have been repeatedly isolated.

The CDC estimates that *E. coli* O157:H7 causes approximately 73,000 illnesses and 61 deaths each year in the US and non-O157 STEC account for an additional 37,740 cases with 30 deaths [1]. Eighty-five percent of these cases are attributed to foodborne transmission. Human infection with

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STEC can result in non-bloody diarrhea, bloody diarrhea, and more serious and potentially fatal complications such as hemorrhagic colitis and hemolytic uremic syndrome (HUS). The most important virulence factors associated with STEC infection include the production of one or more lethal cytotoxins, referred to as Shiga toxins (Stx1, Stx2, or variants) [4]. In addition to toxin production, a number of other potential virulence factors have also been described [5].

The diarrheal phase of illnesses associated with STEC is mostly self-limiting, and the role of early antimicrobial therapy in the prevention of HUS is still unclear. Several published studies focusing on the effect of antimicrobial treatments on STEC infections have been criticized and appear to be controversial [3]. The major concern regarding human antimicrobial treatment of STEC illness is that such treatment may worsen the disease by inducing the release of Shiga toxin(s) and enhance the transfer of virulence factors in vivo [6,7].

However, a recent study of a 1996 large outbreak of *E. coli* O157:H7 in Japan revealed that antibiotic therapy (fosfomycin) significantly reduced the number of infected children that developed HUS [8]. Several reports have also indicated that some antibiotics do not stimulate Shiga toxin release in vivo. Additionally, with the development and approval of 'toxin sponges; i.e. Synsorb Pk®, Synsorb Biotech. Inc., Calgary, AB', which are designed to prevent the progression to HUS in children infected with STEC, some antimicrobials may become routinely administered in the future to help treat STEC related illnesses.

One major complication to this futuristic treatment strategy is the increasing isolation of STEC strains that are resistant to multiple antimicrobials [5,9,10]. Previous research indicated that strains of *E. coli* O157:H7 were susceptible to many different antibiotics [9]. However, several recent studies have now documented antibiotic resistance development among *E. coli* O157:H7 isolates [5,9,10].

Early clinical isolates of *E. coli* O157:H7 were susceptible to many antibiotics. Several studies demonstrated that antibiotic resistance was uncommon within this serotype. In one report, only five (2.9%) of 174 strains of *E. coli* O157:H7 demonstrated resistance to an antibiotic [10]. Additionally, only two strains (1%) of 200 O157:H7 isolates susceptibility tested by the CDC between 1983 and 1985 were resistant to at least one antibiotic [11]. However, the *E. coli* O157:H7 strain responsible for a 1989 waterborne outbreak in Missouri was shown to be resistant to streptomycin, sulfisoxazole, and tetracycline [12]. Another study from Washington State examined human fecal samples collected between 1989 and 1991. This report showed that 13 (7.4%) of 176 strains characterized were resistant to the same three antibiotics [9]. More recently, it was reported that 30 (24%) of 125 strains of *E. coli* O157:H7 and O157:NM isolated from animals, foods and humans displayed resistance to at least one antibiotic and 24 (19%) were resistant to three or more antibiotics [13]. The most frequent resistance phenotype of *E. coli* O157:H7 and O157:NM isolates reported was to streptomycin–sulfisoxazole–tetracycline, which accounted for over 70% of the resistant strains. Additionally, two of these *E. coli* O157:NM isolates from cattle were resistant to six antibiotics: ampicillin, kanamycin, sulfasoxazole, streptomycin, tetracycline and ticarcillin. In fact, increasing resistance to fosfomycin, which is the drug of choice for pediatric gastrointestinal infections due to STEC in Japan, has also been documented [14].

Non-O157 STEC isolated from humans and animals have also developed antibiotic resistance phenotypes, and many are resistant to multiple antimicrobials commonly used in human and veterinary medicine. Schmidt et al. determined antimicrobial susceptibilities among 166 human STEC strains [5]. Nineteen of the 166 STEC strains analyzed displayed resistance to at least one antimicrobial tested, whereas seven strains exhibited resistance to four or more antimicrobials.

The development and dissemination of antimicrobial resistance among STEC strains has not only clinical but also important epidemiological implications. Since cattle are thought to be the main reservoir of STEC and antibiotics are not currently recommended for treatment of *E. coli* O157:H7 infection in humans, the emergence of resistance in this pathogen may be due to the agricultural use of antimicrobials. The emergence of antimicrobial-resistant STEC strains may allow this pathogen to preferentially colonize cattle over other enteric bacteria when antimicrobial selective pressures are present, leading to an increased prevalence in cattle. This could ultimately result in greater contamination of food with STEC. Although the role of antibiotics in treatment of human STEC infections has yet to be defined, it is important that resistance in STEC isolates be actively monitored and investigated.

3. *Salmonella*

The genus *Salmonella* currently includes more than 2400 different serotypes. *Salmonella* species are ubiquitous in the environment and can colonize and cause disease in a variety of food-producing and non-food-producing animals. Salmonellosis in humans caused by non-typhoidal *Salmonella* strains usually results in a self-limiting diarrhea that does not warrant antimicrobial therapy. There are occasions, however, when these infections can lead to life-threatening systemic infections that require effective chemotherapy [15].

Multidrug-resistant phenotypes have been increasingly described among *Salmonella* species on a worldwide basis [15,16]. For example, a recent 7-year study in Spain revealed that ampicillin resistance in *Salmonella* species increased from 8 to 44%, tetracycline resistance from 1 to 42%, chloramphenicol resistance from 1.7 to 26%, and

nalidixic acid resistance from 0.1 to 11% [17]. A similar observation reported increased rates of resistance in Great Britain where resistance in *S. typhimurium* more than doubled between 1981 and 1989 [16]. In the US, resistance to tetracycline in *Salmonella* species has increased from 9% in 1980 to 24% in 1990 and ampicillin resistance increased from 10 to 14% [15]. Fluoroquinolones and expanded-spectrum cephalosporins have become the treatment of choice in the cases of life-threatening salmonellosis due to multidrug-resistant strains [18]. However, there have been increasing reports describing decreased susceptibilities to these antimicrobial agents among *Salmonella* species [18,19].

Although it is possible for salmonellae to spread through person-to-person contact and by human contamination of processed food, the vast majority (95%) of 1.4 million cases estimated to occur annually in the US are attributed to foodborne transmission [1]. Food derived from animals has been directly implicated in numerous *Salmonella* outbreaks over the years. Human cases are often attributed to infection with *S. typhimurium*, one of the most prevalent serotypes in animals. Outbreaks of human salmonellosis directly traceable to contact with farm animals have been reported from as early as the 1960s [20]. There have been many reports since these initial descriptions confirming transmission of *Salmonella* to humans from direct contact with animals. Most recently, an article by Fey et al., reported that a ceftriaxone-resistant isolate of *S. enterica* serotype Typhimurium recovered from a child with diarrhea was indistinguishable from a cattle isolate [18].

An important aspect in this observed decrease in antimicrobial susceptibility to multiple antimicrobial agents in *Salmonella* species is due to the emergence and spread of multidrug-resistant *S. typhimurium* DT104 [21]. Over the past 10 years, the incidence of human infections with multidrug-resistant *S. typhimurium* DT104 has increased dramatically. This organism has spread through food animals to man and has resulted in an increase in resistant *Salmonella* isolates obtained from human infections [21]. A distinctive feature associated with most DT104 isolates is a multiple antibiotic resistance phenotype to ampicillin, chloramphenicol/florfenicol, streptomycin, sulfonamides, and tetracycline (ACSSuT). However, not all DT104 isolates^{-} exhibit this penta-resistant phenotype. Antibiotic-susceptible DT104 isolates have previously been identified from human infections from the 1960s in England. In 1984 the first penta-resistant DT104 isolates were reported associated with sea gulls, then with cattle and then humans in England. The origin of this strain was traced to gulls and exotic birds imported from Indonesia and Hong Kong [21]. Since then, multi-resistant DT104 isolates have been isolated from multiple animal species including poultry, cattle, pigs, sheep, and wild birds [22]. Additionally, DT104 isolates in Great Britain have also acquired resistance to trimethoprim and aminoglycosides and demonstrated decreased susceptibility to fluoroquinolones [23]. The majority of multidrug-resistant DT104 isolates possess a unique chromosomal gene cluster that encodes for the complete spectrum of the ACSSuT resistance phenotype [21].

It is not known whether the spread of DT104 is due primarily to the use of antimicrobials in the animal production environment or via clonal dissemination of a successful pathogen, or both. Regardless, the emergence of antimicrobial resistance in zoonotically transmitted *Salmonella* species is an unfavorable, but perhaps expected, outcome of antimicrobial use in the animal-production environment. The increasing incidence of antimicrobial resistance among *Salmonella* species globally is indeed challenging and it is imperative that we learn more about these resistance phenomena and how to limit their negative effects if we want to assure the continued safe and effective use of antimicrobials in veterinary and human medicine.

4. *Campylobacter*

Campylobacters are thin, curved, motile Gram-negative rods. Members of the genus are generally microaerophillic, although some strains grow aerobically or anaerobically. *Campylobacter* was first generally accepted as an important fecal pathogen in the 1970s, when improvements in culture methods made systematic studies of diarrheal disease feasible. Today, it is recognized as the leading cause of foodborne gastroenteritis in the US and one of the most frequent causes of acute bacterial enteritis worldwide [1].

Campylobacter gastroenteritis is an acute diarrheal disease indistinguishable from those caused by *Salmonella* and *Shigella*. A definitive diagnosis requires isolating the organism from the stools of affected individuals. The disease typically presents with high fever, abdominal cramping, and diarrhea that last from several days to more than one week. Bacteremia and other extraintestinal infections are uncommon, occurring primarily in the young, elderly, and immunocompromised. Severe sequelae, such as Guillain-Barré syndrome, hemolytic uremic syndrome, pancreatitis, and reactive arthritis, affect a small minority of patients. Antibiotic therapy is not necessary in most cases, because the symptoms typically subside after a few days, before a definitive diagnosis can be made and after antimicrobial therapy is most effective. Treatment is typically supportive, consisting of fluid and electrolyte replacement. If laboratory confirmation of *Campylobacter* infection is made, then erythromycin is the drug of choice for uncomplicated cases of enteritis. Otherwise, fluoroquinolones are often given since they are the drugs of choice for the empiric treatment of Gram-negative bacterial enteritis.

C. jejuni and *C. coli*, which are clinically indistinguishable, are the most common species associated with diarrheal illness, causing more than 95% of campylobacter enteritis. Most infections are sporadic single cases resulting from the consumption of contaminated food, milk or water. Undercooked or mishandled poultry appears to be the most

important sources of infection [24]. Large outbreaks are rare and have been linked to ingestion of contaminated milk and water [24]. Even though the infective dose for humans has been estimated as low as 500 bacteria, campylobacter does not survive long when exposed to air and drying. This may explain why foodborne outbreaks are uncommon.

In food-producing animals such as cattle, poultry and swine, fecal *C. jejuni*/*C. coli* is regarded as a commensal organism. *C. jejuni* is routinely recovered from retail chicken products and poultry-processing plants. A 1986 Washington State study showed that 57% of poultry-processing plant samples and 23% of retail chickens carried *C. jejuni* [25]. In Minnesota, surveys of retail chicken products found *Campylobacter* spp. in 88% of 91 chicken meats [26]. Fluoroquinolone-resistant strains were isolated from 20% of the products, with 96% of isolates displaying a ciprofloxacin MIC ≥ 32 g/ml. Other recent surveys have found contamination rates of retail chicken approaching 100% [27].

C. jejuni and *C. coli* are generally susceptible to a variety of antimicrobial agents; however, increasing resistance to some drugs has been documented. A Canadian study showed that resistance to tetracycline increased in *C. jejuni* isolates from 19.1 to 55.7% between 1985 and 1995 [28]. More than 12% of 1995-1997 isolates were resistant to quinolones (nalidixic acid and ciprofloxacin), whereas all isolates from 1985 to 1986 were susceptible to these agents. Among the *C. jejuni* isolates of human origin tested through the US National Antimicrobial Resistance Monitoring System (NARMS) in 1999, 54% were resistant to one or more antimicrobial agents, and 20% were resistant to two or more agents (http://www.cdc.gov/narms/annuals.htm). The most common resistance phenotypes observed were to tetracycline (46%), nalidixic acid (20%) and ciprofloxacin (18%). Among the *C. coli* isolates, 50% were resistant to one or more antimicrobials, and 35% were resistant to two or more agents. The most common resistance among the *C. coli* isolates was to nalidixic acid (30%), followed by tetracycline and ciprofloxacin (30% each).

In the US, there are no reports of ciprofloxacin-resistant human *Campylobacter* isolated prior to 1992. The incidence of fluoroquinolone resistance among NARMS isolates in 1999 was 18%, up from 13% in 1997. This increase coincided with the licensing of fluoroquinolones for treating colibacillosis in poultry, leading to concerns that the association was causal. A similar association was seen in the Netherlands, where the emergence of fluoroquinolone-resistant human *C. jejuni* infections followed the advent of poultry use in 1987 [29]. In Minnesota, the annual percentage of quinolone-resistant infections increased from 1.3% in 1992 to 10.2% in 1998. In 1997, 2 years after the licensing of sarafloxacin in 1995, resistance among *Campylobacter* in Minnesota doubled. Although foreign travel accounted for some of this increase, acquisition of resistant strains from poultry was identified as an important factor [26]. In other countries where antimicrobials are less restricted, fluoroquinolone-resistant *Campylobacter* is a bigger problem. In a report from Spain, 88% of *Campylobacter* isolates displayed fluoroquinolone resistance [30].

The high level of fluoroquinolone-resistant campylobacter in humans and poultry has prompted the FDA, Center for Veterinary Medicine (CVM) to announce in October 2000 a proposal to withdraw approval of the new animal-drug application for use of the fluoroquinolones in poultry. Among other factors, they cited the temporal association between the approval of these drugs for use in poultry in the US and the increase in resistant *Campylobacter* infections in humans. Other evidence supporting this regulatory shift came from surveillance programs, published literature, risk assessment and other sources showing that the use of fluoroquinolones in poultry results in fluoroquinolone-resistant *Campylobacter* infections in humans that can compromise antimicrobial therapy.

5. *Listeria monocytogenes*

L. monocytogenes has been recognized as an important foodborne pathogen since the early 1980s, although the first human case of listeriosis was reported in 1929 [31]. The organism is widely distributed in nature and can be found in soils, decaying vegetation, animal feces, sewage, silage, and water and is frequently carried in the intestinal tract of humans and animals including cattle, poultry, and pigs. *L. monocytogenes* grows well at refrigeration temperatures and minimal nutrients, and is able to survive and even multiply in plants, soil and water. Its widespread nature allows easy access to food products during various phases of production, processing, manufacturing, and distribution. *L. monocytogenes* has been found in a variety of food products, including fresh vegetables, raw meats, raw milk, dairy products, eggs, and seafood products, and epidemic illnesses have largely been linked to refrigerated processed (ready-to-eat) foods consumed without prior cooking or reheating. Examples of high-risk ready-to-eat foods are coleslaw, pasteurized milk, soft cheeses, pâté, pork tongue in jelly, shrimp, and smoked mussels.

The incidence of listeriosis has increased over the past two decades throughout the world. Several large foodborne outbreaks of listeriosis were reported in numerous countries, including England, Germany, Sweden, New Zealand, Switzerland, Australia, France, and the US [31]. It is estimated that in the US each year there are 2500 cases with 500 deaths attributed to listeriosis. A recent outbreak associated with hot dogs in New York involved more than 100 cases with 16 deaths. The spectrum of listeriosis is broad, ranging from asymptomatic infection and carriage to uncommon cutaneous lesions and flu-like symptoms, to miscarriage, stillbirth, septicemia, encephalitis, and meningitis. Although listeriosis can occur in otherwise healthy adults and children, immunocompromised individuals, including the immunosuppressed, the elderly, newborns, pregnant women,

and persons suffering a range of underlying diseases are primarily at risk, with mortality as high as 46%. Foodborne infection with *L. monocytogenes* also can cause febrile illness with gastroenteritis in immunocompetent persons [32].

Ampicillin, rifampin, or penicillin plus gentamicin remain the treatment of choice for most manifestations of listeriosis. Co-trimoxazole is considered to be a second-choice therapy [33]. Vancomycin and erythromycin are also used, respectively, to treat bacteremia and pregnant women diagnosed with listeriosis. In general, most *Listeria* isolates from clinical as well as foodborne and environmental sources are susceptible to the antibiotics active against Gram-positive bacteria. Poulsen et al. [34] reported that 156 strains of *L. monocytogenes* from humans between 1958 and 1985 were susceptible to 12 antibiotics. Comparison of susceptibility patterns between strains isolated in different years showed that antimicrobial susceptibility of *L. monocytogenes* did not change during the last 25 years. However, antibiotic resistance of *Listeria* has emerged. The first *L. monocytogenes* strains resistant to antibiotics were reported in 1988 [35]. Since then, *Listeria* spp. isolated from food, the environment or in sporadic cases of human listeriosis have been shown to be resistant to one or several antibiotics. Charpentier et al. [36] screened 1100 *Listeria* spp. (60 from human patients and 1040 from food and environmental samples) collected worldwide and found many of them to be antibiotic resistant. Of the 61 tetracycline- and minocycline-resistant strains (37 *L. monocytogenes*), 57 harbored the tetracycline-resistance gene *tet* (M) and four non-*L. monocytogenes* isolates contained *tet* (S). Three clinical isolates of *L. monocytogenes* were resistant to low levels of streptomycin, and one strain of *L. monocytogenes* was trimethoprim-resistant.

Further study on the trimethoprim-resistant strain showed that the trimethoprim- resistance gene *dfr*D was located on a 3.7-kb plasmid (pIP823), which belonged to the family of rolling-circle replicating plasmids, common in staphylococci [35]. The *dfr*D gene of *L. monocytogenes* could have originated in the genus *Staphylococcus*. It was found that pIP823 had a broad host range, including *L. monocytogenes*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis*, and *E. coli*. In all these species, pIP823 replicated by generating single-stranded DNA and was stable. Conjugative mobilization of pIP823 was obtained by self-transferable plasmids between *L. monocytogenes* and *E. faecalis*, between *L. monocytogenes* and *E. coli*, and between strains of *E. coli*, and by the streptococcal conjugative transposon Tn*1545* from *L. monocytogenes* to *E. faecalis* and from *L. monocytogenes* to *E. faecalis* to *E. coli*. The findings indicated the gene flux observed in nature from Gram-positive to Gram-negative bacteria could occur by conjugative mobilization. The emergence of trimethoprim resistance in *L. monocytogenes* is of particular importance because the trimethoprim-sulfamethoxazole combination is a successful alternative treatment for human listeriosis.

Poyart-Salmeron et al. [37] reported that 24 of 25 clinical isolates of *L. monocytogenes* carried the *tet* (M) gene associated with a conjugative transposon, and that *tet* (L) was detected in one strain where it was carried on a 5-kb plasmid. Conjugation studies indicated that two types of movable genetic elements, transposons and plasmids, in enterococci and streptococci were responsible for the emergence of drug resistance in *L. monocytogenes*. Multiple antibiotic-resistant strains of *L. monocytogenes* isolated from patients suffering from prosthetic valve endocarditis were reported by Hadorn et al. [38]. Two of these isolates were resistant to chloramphenicol, macrolide/lincosamide/streptogramin and tetracycline. The resistance determinants were located on a 39-kb plasmid, pWDB100, that was transferable to several Gram-positive bacteria, and showed homology to other known resistance genes, *cat221/cat223*, *ermB* and *tet* (M), which are frequently found in different Gram-positive bacteria. Plasmid pWDB100 had a high homology to broad-host-range plasmid pIP501 in *Streptococcus agalactiae* and was also very similar to two *Listeria* plasmids identified in France. Although isolated in geographically different regions, the similarity may illustrate the spread of a plasmid and its relatives. Erythromycin-resistant strains of *L. monocytogenes* isolated from food have been reported [39]. The *ermC* resistance gene was identified and transferred via conjugation to recipient strains of *L. monocytogenes*, *Listeria invanovii* and *E. faecalis*, but did not appear to be associated with conjugative plasmids. Additionally, the vancomycin resistance gene *vanA* has successfully been transferred from enterococci to *L. monocytogenes* and to other *Listeria* spp. by in vitro conjugation [40].

Resistance to other antibiotics in *L. monocytogenes* has also been reported. A strain resistant to gentamicin, streptomycin, chloramphenicol and clindamycin was isolated from a neonate who developed meningitis in Greece [41]. Analysis of 98 *L. monocytogenes* isolates in Italy revealed that two strains were resistant to streptomycin, sulfamethoxazole, and kanamycin, one to streptomycin, sulfamethoxazole, kanamycin, and rifampin, and one to the latter four drugs plus erythromycin and chloramphenicol.

In summary, antibiotic resistance of *L. monocytogenes* has emerged. Studies have demonstrated that this pathogen is capable of acquiring antibiotic resistance genes from foreign sources through movable genetic elements such as transposons and plasmids. The common sources of resistance genes for *L. monocytogenes* appear to be enterococci and streptococci. However, resistance genes could also come from other sources since such genes often flux among Gram-positive and Gram-negative bacteria through conjugative mobilization. Emergence and dissemination of antibiotic resistance in *L. monocytogenes* may have significant future clinical implications for the treatment of listeriosis.

6. *Yersinia*

Presently 11 species of *Yersinia* are recognized; however, only three species are of medical importance; *Y. pestis*, the agent of bubonic and pneumonic plague, and *Y. enterocolitica* and *Y. pseudotuberculosis*, both of which can induce severe gastroenteritis. The pathogenic potential of the other eight species of *Yersinia* (*Y. frederiksenii*, *Y. intermedia*, *Y. kristensenii*, *Y. aldovae*, *Y. bercovieri*, *Y. mollaretti*, *Y. rohdei*, and *Y. ruckeri*) remains controversial at this time. Most isolates of *Yersinia* can be serotyped into >50 serotypes according to somatic O antigens; however, only a few serotypes are considered to be enteric and invasive pathogens [45].

Y. enterocolitica has been isolated from many types of clinical and non-clinical specimens worldwide including Europe, Scandinavia and Canada [42]. Many types of animals carry and act as a natural reservoir of the bacterium, especially pigs, rodents, livestock, and rabbits. One study suggested that domestic animals such as dogs might also act as a reservoir for *Yersinia* [43]. The bacterium is transmitted by ingestion of contaminated food (often milk and pork) and water, probably by the fecal-oral route, and perhaps by contact with infected animals [44]. *Y. enterocolitica* causes several diseases, especially in the young, and is also responsible for several uncommon post-infection syndromes. The common infections include enterocolitis, terminal ileitis, mesenteric lymphadenitis (pseudoappendicular syndrome), septicemia, and focal infections in many extraintrestinal sites.

In the USA, outbreaks of *Y. enterocolitica* have been associated with serogroups O:8, O13:a, 13b and O:3 and O:1,2,3 [42,45]. The most recent outbreak of *Y. enterocolitica* in 1989 showed a disturbing trend. Typically phage type 9b is found in US isolates of O:3, but in the 1989 outbreak some O:3 isolates harbored phage type 8, which is common among European O:3 isolates and is associated with secondary autoimmune phenomena [46]. The antimicrobials of choice for treatment of infections caused by O:3 isolates have not been identified, but trimethoprim-sulfamethoxazole, tetracycline, fluoroquinolones, gentamicin, and chloramphenicol have been recommended for use in septicemic patients [17].

Antibiotic susceptibility patterns of *Y. enterocolitica* appear to be biogroup dependent. Strains of biogroup 1B (serogroup O:8) are resistant to carbenicillin, ticarcillin and cephalothin but are generally susceptible to ampicillin [47], whereas strains of biogroup 1A are resistant to amoxicillin/clavulanic acid [48]. Biogroup 3 strains (O:1,2,3 and O:5,27) have been reported as susceptible to carbenicillin and ticarcillin, but show resistance to amoxicillin/clavulanic acid and cefoxitin [49]. Serogroup isolates (O:3) are resistant to carbenicillin, ticarcillin and ampicillin but remain susceptible to amoxicillin/clavulanic acid and cefoxitin [50]. Interestingly, β-lactam resistance is mediated by the production of two chromosomally mediated β-lactamases (A and B). These two β-lactamases account for resistance to ampicillin, cephalothin, carbenicillin, and penicillin [45]. The type A β-lactamase hydrolyzes a variety of penicillins and cephalosporins whilst the type B β-lactamase shows strong cephalosporinase activity. These two β-lactamases can act synergistically to confer resistance to a variety of other β-lactam antibiotics [42].

In contrast to the above, *Yersinia* remain susceptible to tetracycline and quinolones [50]. However, a recent report from Barcelona, Spain compared antibiotic resistance trends in *Y. enterocolitica* O3 strains (biotype 4) between 1985 and 1987 and 1995 and 1998 [17]. All isolates (n = 75) recovered from 1985 to 1987 were resistant to ampicillin and 72% of strains were resistant to streptomycin, 45% to sulfonamides, 28% to trimethoprim–sulfamethoxazole, and 20% were resistant to chloramphenicol. Interestingly, from 1995 to 1998, *Y. enterocolitica* strains demonstrated increased resistance rates to streptomycin (90%), trimethoprim–sulfamethoxazole (70%), chloramphenicol (60%), and nalidixic acid (5%) [17].

Yersinia spp. remain a formidable pathogen armed with an array of resistance genes and plasmid mediated virulence factors. In particular, *Y. enterocolitica* has emerged as an enteropathogen associated with several types of human illnesses that often require antimicrobial therapy. Increasing antibiotic resistance in this pathogen is another developing dilemma that must be monitored closely. Further research is also needed to improve our understanding of the pathogenesis of the disease process and the exact mechanism of its passage from the environment reservoir to its adaptation in the human host.

7. *Summary*

During the past five decades, the use and sometimes misuse of antimicrobials in both human and veterinary medicine have given rise to a selection unprecedented in the history of microbial evolution. As a result, society is facing one of the most serious public health dilemmas ever: the emergence of infectious bacteria displaying resistance to many, and in some cases all, effective antimicrobials. There is currently a great deal of conjecture regarding the role that therapeutic and sub-therapeutic use of antimicrobials in animals has played in accelerating the development and dissemination of antimicrobial-resistant bacterial pathogens.

Much like the situation in human medicine, the use of antibiotics in livestock and poultry species has accelerated the development of antibiotic-resistant strains of microbial pathogens, potentially complicating treatment for both animals and humans. However, there is still no complete accord on the significance of antimicrobial use in animals and the emergence and dissemination of antibiotic resistance among human bacterial pathogens.

The increasing incidence of antimicrobial-resistant bacterial pathogens will have serious repercussions for the future treatment and prevention of infectious diseases in both animals and humans. Although a great deal of scientific information is available on this subject, many aspects of the development and dissemination of antimicrobial resistance in the animal production environment still remain murky. Research in this area has demonstrated that the manifestation and dissemination of bacterial antimicrobial resistance is the result of countless complex interactions between microorganisms, antimicrobials, and the surrounding environments. We must strive to better comprehend these complex interactions if science-based risk assessments concerning the use of antimicrobials in the animal production environment are to be made. Only through a committed approach can we begin to reverse resistance trends and maintain the effectiveness of antimicrobials in human and veterinary medicine.

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