Review Paper

ANTIBIOTIC RESISTANT *SALMONELLA SPP*: MECHANISM OF DRUG RESISTANCE, GENE VARIATIONS AND CLINICAL IMPLICATIONS.

Abstract

Salmonella spp are etiological agents of diarrhea and systemic infections in humans, most commonly as secondary contaminants of food originating from animals and the environment or irrigated by faecal wastes. Decades of indiscriminate use and abuse of antibiotics have resulted in increased development of antibiotic resistance in *Salmonella spp* to different antibiotics, creating major problems in treatment of relapsing salmonellosis and other enteric diseases across many age groups. This review x-rays the mechanisms of antibiotic resistances, genetic variations and clinical implications of antibiotic resistant *Salmonella spp*. The findings of this review revealed that antibiotic resistance in *Salmonella spp* resulted from a wide range of mechanisms developed by serovars of Salmonella. It has also been discovered from scientific studies that the multiple antibiotic resistances noticed in many serovars of Salmonella were due to genetic modifications in these serovars, chiefly mutation. Adequate drug use control and antibiotic combination therapies are encouraged for effective prophylaxis of relapsing salmonellosis caused by antibiotic resistant *Salmonella spp*.

Keywords: Antibiotic resistant Salmonella, Mechanism of resistance, Clinical implications, relapsing salmonellosis, Genetic variations.

Introduction

Salmonellosis is an infectious disease of humans and animals caused by organisms of the genus *Salmonella* (De Oliveira *et al.* 2010). Although primarily intestinal bacteria, *Salmonella* are present in the environment and may commonly be found in farm effluents, human sewage and in any material subject to faecal contamination (Boyen *et al.* 2008). The genus *Salmonella* consists of only two major species: *S. enterica* and *S. bongori* (Grimont and Weill, 2007, Gomez *et al.*, 2010). *Salmonella enterica* is divided into six subspecies, which are distinguishable by biochemical characteristics (Grimont and Weill, 2007, Gomez *et al.*, 2010; De Oliveira *et al.* 2010). These include sub-species *enterica, salamae, arizonae, diarizonae, houtenae* and *indica*. Strains of *Salmonella* are classified into serovars on the basis of extensive diversity of lipopolysaccharide (LPS) antigens (O) and flagellar protein antigens (H) in accordance with the Kauffmann–White scheme; currently over 2500 serovars are recognized (Grimont and Weill, 2007; Gomez *et al.* 2010). Salmonellosis in humans can be treated with a number of antibiotics including ampicillin, amoxicillin, gentamicin, trimethoprim/sulfamethoxazole and fluoroquinolones (Grimont and Weill, 2007, Gomez *et al.*, 2010). Many isolates are resistant to one or more antibiotics, and the choice of drugs should, if possible, be based on susceptibility testing (Gobetti *et al.*, 2007).

Antibiotic-resistant strains of *Salmonella* have been isolated in most endemic areas, particularly Southeast Asia, India, Pakistan and Middle East (Bhunia, 2008; Gomez *et al.*, 2010). Generally, antibiotic resistance in *Salmonella spp* is due mainly to suboptimal use of antibiotics for prophylactic treatment and prolonged hospitalization (Bhunia, 2008). The population of organisms that spontaneously acquire these resistance mechanisms as a result of selective pressure from different antibiotics is growing at an alarming rate (Bhunia, 2008). Some of the

antibiotics the *Salmonella spp* had developed resistance against include penicillin, amino glycosides, tetracycline, cephalosporin, macrolides and ketolides and several others (Ling *et al.* 2002, Gomez *et al.*, 2010).

Genotypic analysis of the antibiotic resistant *Salmonella spp* by use of real time-polymerase chain reaction (RT-PCR) and molecular fingerprinting of DNA has been used to good effect (Foley *et al.*2007). Plasmid gene profile analysis is a quick and relatively easy method to fingerprint strains, and has been used in both human and veterinary medicine to study the spread of antibiotic resistant *Salmonella* (Torpdahl *et al.* 2007). This technique has limitations as not all strains of *Salmonella* have plasmids, and plasmids may be readily acquired or may be of similar size but genetically different (Foley *et al.*2007). Phage typing or alternative genetic techniques and full DNA sequencing is increasingly used to study genetic variations in antibiotic resistant *Salmonella spp* chiefly because of its low cost automated methods (Torpdahl *et al.* 2007).

Mechanisms of Antibiotic Resistance

Antibiotic resistances in *Salmonella spp* can result from enzymatic inactivation, decreased permeability, development of efflux pump systems, alteration of target sites and in most cases in many serovars the overproduction of target sites to overwhelm the used antibiotics (Singh *et al.,* 2010). In several cases investigated, antibiotic resistances can be acquired through natural selection or mutation (induced or spontaneous); this however can be chromosomal mutation by the production of chromosomally mediated inducible enzymes or acquisition of plasmid resistant genes: this been the most common genetic basis of antibiotic resistance (Bhunia, 2008; Gomez *et al.*, 2010 and Singh *et al.*, 2010). Genetic determinants can spread laterally through a population without cell division; this can be

via interspecies lateral transfer of plasmids or resistances usually involving antibiotic inactivating enzymes (many encoded by transposons) (Bhunia, 2008).

In general, the reasons for increasing antibiotic resistance levels in many *Salmonella* isolates include the suboptimal use of antimicrobials for prophylaxis and treatment of infection, non-compliance with infection-control practices, prolonged hospitalization, increased duration of intensive care-unit stays, multiple co-morbidities in hospitalized patients, increased use of invasive devices and catheters, ineffective infection-control practices, transfer of colonized patients from hospital to hospital, grouping of colonized patients in long-term-care facilities and increasing national and international travel (Bhunia, 2008; Gomez *et al.*, 2010 and Singh *et al*., 2010). Furthermore, the level of antibiotic resistance is dependent on the population of organisms that spontaneously acquire resistance mechanisms as a result of selective pressure either from antibiotic use or otherwise (Gomez *et al.*, 2010).

The antibiotic resistance of *Salmonella spp* to a single antibiotic was first reported in the early 1960s (Montville and Matthews, 2008). Since then, the isolation frequency of *Salmonella* strains resistant to one or more antibiotics have increased in the Saudi Arabia, United States, United Kingdom and other countries of the World (Montville and Matthews, 2008; De Oliveira *et al.* 2010). Emerging resistance in *Salmonella typhi* has been described especially in Africa and Asia and the appearance of *Salmonella typhimurium* DT104 in the late 1980s raised main public health concern, thereby threatening the lives of infected individuals stated that multi-resistance occurred in *Salmonella* serotypes including *albany*, *anatum, havana, london andTyphimurium* (Yoke-Kqueen *et al.* 2007; De Oliveira *et al.* 2010).

The resistance towards the traditional first-line antibiotics such as ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole defined multidrug resistance (MDR) in

Salmonella enterica (Gobetti *et al*., 2007; Singh *et al*., 2010). This has been of great concern because majority infections with *Salmonella* are acquired through the consumption of contaminated foods of animal origin such as swine and chicken eggs. In addition, antibiogram testing by Singh *et al.*, 2010 revealed *Salmonella* isolates from chicken eggs in marketing channels and poultry farms in North India were resistant to bacitracin, colistin and polymyxin-B. In addition, there is a need of continuous surveillance and sharing of antimicrobial susceptibility data for *Salmonella* among countries worldwide to ensure the effectiveness of control programmes (De Oliveira *et al.* 2010).

Multiple Antibiotic Resistances in *Salmonella spp*

Multidrug resistance among many serovars of *Salmonella* has become a big challenge to infectious disease management (Dessen et al., 2001). It is increasingly being reported in bacteria and is often mediated by genetic mobile elements such as plasmids, transposons and integrons (Dessen *et al*., 2001, Asai *et al.,* 2010). Integrons are mobile DNA elements with the ability to capture genes, notably those encoding antibiotic resistance, by site specific recombination, and they have an intergrase gene (int), a nearby recombination site (attI), and a promoter. Integrons seem to have a major role in the spread of multidrug resistance in gram-negative bacteria but integrons in gram-positive bacteria have also been described (Dessen et al., 2001, Asai *et al.*, 2010). The majority of genes encode antibiotic resistance, including resistance to aminoglycosides, penicillins, cephalosporins, trimethoprim, tetracycline, erythromycin, and chloramphenicol (Asai *et al.*, 2010).

Clinical Implications of Antibiotic resistant *Salmonella spp*

In human disease, the clinical pattern of salmonellosis can be divided into four disease patterns namely enteric fever, gastroenteritis, bacteremia and other complications of non-typhoidal salmonellosis as well as chronic carrier state (Piu *et al.* 2008).

Enteric Fever

Salmonella enterica Sp.typhi causes typhoid fever whereas *Salmonella enterica Sp.paratyphi* A, B and C cause paratyphoid fever with symptoms which are milder and mortality rate that is lower for the latter (Jalali *et al.*, 2008). Roughly 10% of patients may relapse, die or encounter serious complications such as typhoid encephalopathy, gastrointestinal bleeding and intestinal perforation (Piu *et al*., 2008). Relapse is the most common occurrence chiefly due to persisting infections by antibiotic resistant strains of *Salmonella spp* within reticulo-endothelial system (RES) (Jalali *et al.*, 2008). Typhoid encephalopathy, often accompanied by shock, is associated with high mortality (Jalali *et al*., 2008). Slight gastrointestinal bleeding can be resolved without blood transfusion but 1 to 2% of cases can be fatal if a large vessel is involved (Freitas *et al.* 2010). Intestinal perforation may present with abdominal pain, rising pulse and falling blood pressure in sick people; hence, it is very serious in 1 to 3% of hospitalized patients (Freitas *et al.* 2010).

Gastroenteritis

Non-typhoidal salmonellosis or entero-colitis is caused by at least 150 *Salmonella* serotypes with *Salmonella enterica Sp.typhimurium* and *Salmonella enterica Sp. enteritidis* being the most common serotypes in the United States (Gobetti *et al.*, 2007). Infection always occurs via ingestion of water or food contaminated with animal waste rather than human waste Freitas *et al.* 2010). The emergence of multidrug-resistant *Salmonella enterica Sp .typhimurium* DT104

has been associated with outbreaks related to beef contamination and resulted in hospitalization rates twice than that of other food borne salmonellosis (Gray *et al.* 2002; Freitas *et al.* 2010).

Bacteremia and other complications of non-typhoidal salmonellosis

About 8% of the untreated cases of salmonellosis result in bacteremia. Bacteremia is a serious condition in which bacteria enter the bloodstream after passing through the intestinal barrier (Freitas *et al.* 2010). It has been associated with highly invasive serotypes like Cholearaesuis or Dublin (Boyen *et al.*, 2008). Bacteremia caused by *Salmonella spp* should be taken into account in cases of fever of unknown origin (Jalali *et al.* 2008).

Chronic Carrier State

Salmonellosis can be spread by chronic carriers who potentially infect many individuals, especially those who work in food-related industries (Boyen *et al.*, 2008). On average, non-typhoidal serotypes persist in the gastrointestinal tract from 6 weeks to 3 months, depending on the serotypes (Boyen *et al.*, 2008; Jalali *et al*., 2010). Only about 0.1% of non-typhoidal *Salmonella spp* cases are shed in stool samples for periods exceeding 1 year (Boyen *et al.*, 2008). Up to 10% of untreated convalescent typhoid cases will excrete *Salmonella typhi* in feaces for 1 to 3 months and between 1 and 4% become chronic carriers excreting the microorganism for more than one year (Byarugaba, 2004).

Genetic Variations in Antibiotic Resistant *Salmonella spp*

The study of bacterial genetics has provided much of the conceptual foundation for understanding the structure, function, and expression of genes (Asai *et al.*, 2010). The detailed knowledge of genetic mechanisms of antibiotic resistances in bacteria has also resulted in immensely powerful and sophisticated tools for studying the molecular biology of a wide variety of prokaryotic and eukaryotic organisms (Dessen et al., 2001, Asai *et al.*, 2010). Because many

of these tools make it possible to do detailed genetic studies on previously intractable bacterial species, there has been considerable interest and recent exciting progress in studying the genetic basis of multiple antibiotic resistances, pathogenesis and how different antibiotic resistant isolates differ in genetic features that code for antibiotic resistances noticed in them (Foley *et al*., 2007; Asai *et al.*, 2010). Sometimes, with appropriate selective pressure, new genes and elements can evolve and spread rapidly (Foley *et al*., 2007).

One of the most deadly examples is the development of genetic elements that encode resistance to several antibiotics and transfer easily from one bacterial cell to another (Freitas *et al*., 2010). Such elements have caused severe problems in the treatment of infectious bacterial disease (Asai *et al.,* 2010). In other cases, the genetic changes are programmed by the bacterial cell, as in the case of antigenic variation of certain pathogens are programmed by the bacterial cell, resulting in antigenic variation of certain pathogens (Dessen et al., 2001, Asai *et al.,* 2010). Vertical inheritance by natural selection can occur in certain serovars of *Salmonella* in which occasionally, a spontaneous genetic change occurs in one of the cells (Freitas *et al*., 2010). This change (mutation) is heritable and passed on to the progeny of the variant cell to produce a sub-clone with characteristics different from the original (wild type) parent (Freitas *et al*., 2010). If the change is detrimental to the growth of the cell, the sub-clone will quickly be overrun by the healthy, wild type population; however, if the change is beneficial, the sub-clone may overtake the wild type population in a process of natural selection (Dessen et al., 2001, Asai *et al.*, 2010).

Certain antibiotic resistant isolates or serovars of *Salmonella* may exhibit genetic variations due to spontaneous mutation (Asai *et al*., 2010). This may occur by point mutation, which can be via change of a single nucleotide, DNA rearrangement, or shuffling of the genetic information to produce insertions, deletions, inversions, or changes in structure (Freitas *et al*.,

2010). This particularly may also effect the changes in feeding affinities of some certain serovars of *Salmonella* as some have been reported to develop ability of utilizing lactose sugar (Torpdhal *et al*., 2007; Gomez *et al*., 2010). DNA rearrangements can affect a few to several thousand nucleotides; both types of mutations generally occur at a low frequency and lead to a continuous, slow evolution of bacterial populations (Gray *et al*., 2002; Torpdhal *et al*., 2007).

Bacterial variation can also occur by horizontal transfer of genetic material from one cell to another. This may occur via transformational release and uptake of naked DNA, Transduction; a process of packaging and transfer of bacterial DNA by viruses, and Conjugation; a process of bacterial mating in which cells must be in contact (Torpdhal *et al*., 2007; Asia *et al*., 2010). The transferred DNA is stably incorporated into the genetic material of the recipient bacterium through recombination and integration of the transferred DNA into the bacterial chromosome or establishment of a plasmid i.e., the transferred material essentially forms a mini-chromosome capable of autonomous replication (Torpdhal *et al*., 2007). Although antibiotic resistances to a wide array of antibiotics in some *Salmonella* serovars have also been mediated by the possession of transposons and integrons; mobile DNA elements with the ability to capture genes, notably those encoding antibiotic resistance, by in-site specific recombination and integration of genes coding for antibiotic resistance (Torpdhal *et al*., 2007; Gomez *et al*., 2010).

CONCLUSION

The incidence of relapsing salmonellosis outbreak mediated by several antibiotic resistant serovars of *Salmonella* cannot be neglected due to its overwhelming clinical implications in humans (Torpdhal *et al*., 2007; Jalali *et al*., 2008; Singh *et al*., 2010). As a result, further research studies should be conducted on the transmission vehicles and pathogenesis of these antibiotic resistant serovars to estimate the lethal effects they would have in an apparently

healthy host as compared to the antibiotic susceptible strains and if possible, amplified genetic studies may also be integrated into bacteriological studies for better understanding of the mechanisms developed by the resistant serovars to different antibiotics.

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