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Multidrug-resistant *Salmonella*: A raising calamity

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ABSTRACT

Multidrug-resistant (MDR) organisms become a foremost health concern in both developing and developed countries. These infectious agents show a wide spectrum of resistance against different antimicrobial drugs which causes a major public health threat all over the world. In recent years bacteria, fungi, virus, and parasites started to show prominent levels of multidrug-resistance with superior morbidity and mortality. MDR Salmonellosis requires costly drugs for effective treatment, and this is an added burden to the health-care segment in developing countries. Immunization in common areas with typhoid vaccine, rational use of antibiotic, improvement in public sanitation facilities, secure food handling and communal health instruction were considered to be as vital in the prevention of salmonellosis. These health and environmental issues require collective actions from governments, consumers, pharmaceutical manufacturers and health-care providers. Two methods were followed to congregate the information in this article. Firstly, we collected a list of relevant articles published through PubMed search, which includes original as well review articles. Secondly, book chapters on typhoid fever from standard textbooks on infectious diseases and social remedy were also reviewed.

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INTRODUCTION

"There is probably no chemotherapeutic drug to which; In suitable circumstances, the bacteria cannot react; By in some way acquiring 'fastness' [resistance]."-Alexander Fleming, 1946.

Salmonella is a genus of Gram-negative facultative anaerobe and flagellated rod-shaped bacterium which belongs to the family of *Enterobacteriaceae*, cell diameters ranging around 0.7 and 1.5 µm, lengths from 2 to 5 µm (Kanungo *et al.,* 2008). It continues to be a universal public health crisis with over 21.6 million cases and 250,000 deaths annually (Kothari *et al.,* 2008; Srikantiah *et al.,* 2006). More than 80% of salmonellosis was reported particularly in Asia, and the remaining was in Africa and Latin America (Nagshetty *et al.,* 2009). Among the developing countries particularly in India, the occurrence of disease is ranging from 10 to 2,200 per 10,000 of the population (Chowta *et al.,* 2005). A number of studies in these areas have shown about one-third of pediatric typhoid cases are under five years of age (Lin *et al.,* 2000). Neonates, infants and older children are highly susceptible to salmonellosis with common symptoms such as septicemia, diarrhoea, and lower respiratory tract infections (Mohanty *et al.,* 2009; Karande *et al.,* 1995; Olutola *et al.,* 1985). There is no effective vaccine against MDR salmo-

nellosis, and this phenomenal character may easily target a child below the age of two.

Epidemiology

Salmonellosis is stable in both developing and developed countries. Typhoid or enteric fever usually causes mortality in 10 to 30% in the developing world further World Health Organization (WHO) calculates approximately 15 to 17 million cases of salmonellosis occurs annually. The incidence of death rates vary from one province to other, but it can be as high as 5 to 7% even though the use of suitable antibiotic treatment. Data on salmonellosis are inadequate in many countries such as Asia, Africa, South and Central America (Miliotis *et al.,* 2003). Some of the incidence, notification, and seclusion pace of salmonellosis in a different part of the world are shown in Figure 1. The infectious dose of *Salmonella* may depend upon the strain, serovar, and host susceptibility. On the other hand, factors such as intestinal tract, age and underlying illness or immune deficiencies of the host were also considerable. The infectious dose of *Salmonella* is varying from 1 to 9 cfu/g. However, single-food-source with 1 to 10 cells can cause salmonellosis with more susceptibility to young, old, pregnant and immune suppressed (YOPI) groups (Bhunia, 2008).

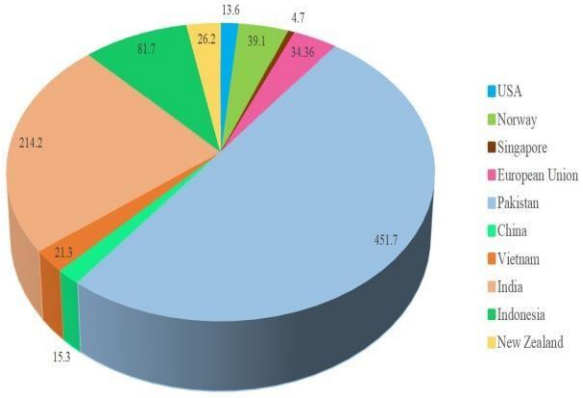

Figure 1: Incidence, and seclusion pace of salmonellosis in a different part of the world

Transmission

Salmonella spp. are broadly scattered in the environment, and they subsist fine in a diversity of food sources like dairy products, poultry crop, eggs foodstuffs which are the universal vehicles. Recently fresh fruits and vegetables also gained particular microbe *Salmonella* at multiple steps beside with food chains (Bouchrif *et al.,* 2009). Initially, the contaminated atmosphere serves up as the infection source of *Salmonella*, and this can be alive for several weeks. Next, the infectious agent can be transmitted to vectors (rat, birds, and swine) where *Salmonella* is shed in their faeces for weeks even month. Finally, humans acquire infection through drinking the water or eating the food which is contaminated by *Salmonella* through animal reservoirs (Newell *et al.,* 2010).

MDR *Salmonella*

A further complication of salmonellosis was obtained with the emergence of MDR *Salmonella* in the last two decades and spreading worldwide, resulting in high rates of morbidity and mortality (Gupta , 1994; Memon *et al.,* 1997; Saha *et al.,* 2006; Kumar *et al.,* 2007; Bavdekar, 1996). MDR *Salmonella* can be defined as *Salmonella* which shows resistant to a wide spectrum of antibiotics. General effective antibiotics consider for *Salmonella*e such as ampicillin, chloramphenicol, and co-trimoxazole (Kato *et al.,* 2007; Kumar *et al.,* 2008). In the past decade, a major number of antibiotics become unsuccessful for the treatment of *Salmonella* considerably. The appearance of multidrug-resistance further complicates the treatment and management of enteric fever, and this can be predicted as one of the furthermost challenge (Sehra *et al.,* 2013). The idea of this review is to expound about the organization, taxonomy, feature, clinical appearance, epidemiology, vehicles transmission and antibiotic resistance pattern of *Salmonella*.

At present, more than 107 strains of *Salmonella* spp. and over 2000 serotypes of *Salmonella* infects human which have been isolated with different metabolic characteristic, levels of virulence and multi-drug resistance genes which are further complicating the treatment (Parkhill *et al.,* 2001; Deng *et al.,* 2003). Investigative detection can be attained by growth on MacConkey and Eosin Methylene blue agar. *Salmonella* is strictly non-lactose fermenting and also produces no gas when grown in triple sugar iron agar, this may use to differentiate it from other *Enterobacteriaceae*. Even though, the newer antibacterial drugs appearing, Salmonellosis has continued to be a major health problem (Zaki *et al.,* 2011).

Antimicrobial resistance & global dominance

In 1947 chloramphenicol was discovered and has been used to treat typhoid fever caused by *Salmonella*. With the emergence of plasmid-mediated chloramphenicol resistant *S.typhi* throughout the world, there is a need for a newer antibiotic with good *in vivo* activity against *S.typhi*. Ciprofloxacin, ofloxacin, and perfloxacin are most extensively used antibiotics inhibit DNA gyrase which plays a vital role in coiling and supercoiling of bacterial DNA during replication. The third generation antibiotics such as cefoperazone, cefotaxime, and ceftriaxone are used effectively as a therapeutic alternative for multidrug-resistant *S.typhi* cases (Arora *et al.,* 2011). While a strain gains resistance to a particular drug, there must be anoth-

er drug to be found as a therapeutic agent. However if the bacteria shows resistant to the second drug, a third drug should be in need (Black, 2005).

The first multidrug-resistant strains were reported in Southeast Asia in the year of 1980s (Mirza *et al.,* 1996). Reports of MDR in Asian countries include China(1985), Pakistan(1987), India (1988), Malaysia (1991), Singapore (1994), Bangladesh (1994), Vietnam (1995), Japan (1999), Thailand (2001), Korea (2003), Nepal (2005) and Indonesia (2009); other countries include Kuwait (1996) and Jordan (2008) (Swaddiwudhipong, 2001; Pai *et al.,* 2003; Lewis *et al.,* 2005; Yanagi *et al.,* 2009). In a very recent multi-centric study conducted across five Asian countries (China, India, Indonesia, Pakistan, and Vietnam) for typhoid and the occurrence of MDR ranging from 7% to 65% were reported (Ochiai *et al.,* 2008). Other than Asian countries, MDR also reported in African countries including South Africa (1992), Kenya (2000), Nigeria (2005) and Egypt (2006) (Srikantiah *et al.,* 2006; Kariuki *et al.,* 2000; Akinyemi *et al.,* 2005; Coovadia *et al.,* 1992). Even developed countries like the United Kingdom (1990), America (1997) and Italy (2000) also reported for MDR prevalence. Most cases were found among travellers who frequently cross countries (Scuderi *et al.,* 2000; Misra *et al.,* 1997). Earlier studies from different countries have reported the presence of multidrug-resistant typhoid fever (MDRTF) (Yoo *et al.,* 2004; Dimitrov *et al.,* 2007; Naheed *et al.,* 2010; Mengo *et al.,* 2010; Marks *et al.,* 2010; Lynch *et al.,* 2009).

MDR in India

In India, the first MDR *Salmonella* was first reported from Mumbai in the year 1988, and it has been spread throughout the country (Das *et al.,* 2000; Sen *et al.,* 2007; Madhulika *et al.,* 2004; Jog *et al.,* 2008; Arora *et al.,* 2009; Renuka *et al.,* 2004; Mishra *et al.,* 1991). In the 1990s, a clinical study in Bangalore reported the high resistant *Salmonella* strain against ampicillin, co-trimoxazole, and chloramphenicol which were reported as 95% resistant pattern (Rathish *et al.,* 1995). The mid of 1999, similar findings of high resistance against the drug as mentioned above were also reported from Manipal (Ciraj *et al.,* 1999). Though ciprofloxacin had a high cure rate, it was believed to be the best preference for the treatment of multidrug-resistant typhoid cases. In the next few years, studies from different parts of India disproved that ciprofloxacin is the best choice against MDR *Salmonella* (Kumar *et al.,* 2008). At the beginning of the twentieth century, a study was done by Das *et al*. in Orissa reported around 2.5% of *S.typhi* strains showed higher resistant against ciprofloxacin (Das *et al.,* 2000). A similar study in Delhi also reported that the occurrence of MDR gradually increased from 34% to 66% from the year 1999-2005 (Kumar *et al.,* 2008). Another few similar studies reported the gradual development of resistance against fluoroquinolone. Later, several isolated reports of ceftriaxone-resistant *S.typhi* strains were reported in different parts of India (Nagshetty *et al.,* 2009; Kumar *et al.,* 2008). These observations of increasing resistance to ciprofloxacin and early evidence of resistance to ceftriaxone correspond with the global picture (Saha *et al.,* 2006; Yoo *et al.,* 2004).

MDR in *S. Typhi*

Generally *Salmonella* spp. exhibit drug resistance via plasmid-mediated mechanism and or chromosomal DNA-mediated mechanism (Gupta *et al.,* 1994; Memon *et al.,* 1997; Al-Sanouri *et al.,* 2008; Malik, 2002; Rowe *et al.,* 1997). Plasmids are covalently closed circular DNA which carries the drug-resistant gene (Hawkey *et al.,* 1998). The chromosomal-mediated drug resistance against fluoroquinolone was recognised as a single point mutation in the quinolone resistance determining the region (QRDR) of the topoisomerase gene *gyrA.* (Renuka *et al.,* 2004; Phan *et al.,* 2008; Turner *et al.,* 2006). Some other mechanisms such as efflux pumps and decreased permeability of drug were also described (Bhutta, 2006) (figure 2).

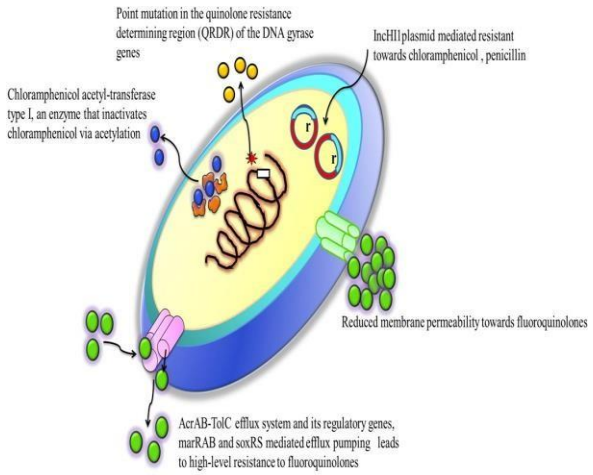

Figure 2: Antimicrobial resistance Mechanism of Salmonella spp

Antimicrobial resistance of *Salmonella* can be expressed by 4 major mechanisms.

I. Inactivation of the antimicrobial agent by the enzyme.
II. Reduction of membrane permeability
III. Modification of the target site.
IV. Efflux or transport of the antimicrobial drug.

Major drug resistance may be due to changes in the genome, either a chromosomal mutation or the gaining of a plasmid (Denyer *et al.,* 2011).

Plasmid-mediated resistance

Plasmid-mediated resistance habitually codes an enzyme that may destroy or modify drugs. Plasmid-mediated resistance has been reported against aminoglycosides, chloramphenicol, penicillin, cephalosporin, tetracycline, sulfonamides (Willey *et al.,* 2013).

However, antibiotic resistance in Salmonella is frequently plasmid mediated. HI1 Plasmid vector plays a vital role in antibiotic resistance in Salmonella (Phan *et al.,* 2008; Turner *et al.,* 2006). In the year 1972, the first InchI1 plasmid was isolated and reported in S.typhi from a large outbreak of resistant typhoid fever in Mexico City. Also, they found MDR S.typhi showed resistance against tetracycline, chloramphenicol, streptomycin, and sulfonamides. Consequently, the MDR strain of S.typhi was spread globally. With the emergence of these MDR, S.typhi strains have become a grave problem globally and have been reported (Shanahan et., 1998).

The mechanism of drug resistance usually mediated by acquisition of R plasmids involves:

Inactivation of the drug

Inactivation or destruction of the antimicrobial agent is a general ground of resistance. The pathogens may show resistance against the attack of antimicrobial agent through by inactivating drugs via chemical modification. β-lactamase is one of the familiar enzymes exist in various bacteria which categorise under this section. This enzyme easily hydrolyzes the β-lactam ring which is present in major antibiotics. The initial strains of antibiotic-resistant S.typhi carried chloramphenicol acetyltransferase type I, which encodes an enzyme that inactivates chloramphenicol via acetylation (Zaki *et al.,* 2011). Chloramphenicol contains two hydroxyl groups that can be acetylated in a reaction catalyzed by the enzyme chloramphenicol acetyltransferase with acetyl CoA as the donor. Aminoglycosides can be modified and inactivated in several ways. Acetyltransferase catalyzes the acetylation of amino groups. Some aminoglycoside modifying enzymes catalyze the addition of either phosphate (phosphotransferase) or adenyl groups (adenyltransferase) to hydroxyl moiety (Black, 2005; Willey *et al.,* 2013).

Reduction in membrane permeability

Multidrug-resistant strains frequently become resistant via preventing entrance of the drug. Modification of genome can change the wild nature of proteins that modify the membrane permeability. Such alterations change a membrane selective transportation system; ultimately therapeutic drug can no longer cross the membrane. By this mechanism, *S.typhi* exhibit resistance to tetracycline, quinolones, and some aminoglycosides (Black, 2005; Willey *et al.,* 2013).

Modification of target site

Commonly antimicrobial agents are equipped with specific target sites. In MDR, resistance occurs when the target enzyme or cellular structure of the pathogen is modified so that it is no longer susceptible to the drug. This mechanism is found in S. Typhi and other sulfonamide-resistant bacteria. They have developed an enzyme that has a high affinity for p-aminobenzoic acid (PABA) and a very low affinity for sulfonamide. As a result, even in the presence of sulfonamides, the enzyme works well enough to allow the bacterium to function (Black, 2005).

Rapid efflux of the antimicrobial agent

After the entry of the drug into the cell, there may be the possibility of pumping out the drug from the cell. Some pathogens like Salmonella have plasma membrane efflux pumps that are used to expel drugs after its entry into the cell. Though they are nonspecific, it can easily pump out various drugs, so it can be called multidrug-resistance pumps. Resistance to sulfonamides is mediated by a plasmid-encoded transport system that actively exports the drug out of the cell (Denyer *et al.,* 2011). Many genes, such as plasmid-mediated β lactamase, tetracycline-resistance, and aminoglycoside-modifying enzymes are organized on transposon (Richard *et al.,* 2007).

Chromosome-mediated- resistance

Chromosomal mediated resistance has been known by a point mutation in the gene that encodes for a target of the drug that prevents the penetration of the drug (Denyer *et al.,* 2011). Commonly it has been known that chromosomal mediated resistance is less important than plasmid-mediated because the frequency of spontaneous mutation in chromosomes is usually in the ranges from 10-7 to 10-9.

Cause and factors for MDR in S. Typhi

Causative factors for the development of resistance in S. typhi include overuse, maltreatment, and unsuitable antimicrobial prescribing practices (Tunger *et al.,* 2009). Time pressures and diagnostic qualms are some of the main reasons behind illogical prescription. Further allopathic drugs by nonlicensed practitioners such as homoeopaths, unani and ayurvedic practitioners aggravate the complication. Huge application of antibiotics in agriculture, animal husbandry and fisheries have further provoked the problem of MDR. In many developing countries like India, there is no proper monitoring and quality control

system for local antimicrobial drug production. Most of the time this may have uncertain quality and potency control, coupled with poor economics of patients to costly antimicrobials adds to the threat of antimicrobial resistance (Farrar *et al.,* 2001; Shanahan *et al.,* 1998; Richard *et al.,* 2007; Tunger *et al.,* 2009; Sharma *et al.,* 2005; Hart *et al.,* 1998). Though common cold, cough, and diarrhoea can be easily resolved by our immune system, antibiotics are unnecessarily prescribed. Also in many cases, treatment with broad-spectrum antibiotics has been rushed over before finding the causative organism, and this leads to the emergence of MDR organism (Tunger *et al.,* 2009).

Diagnosis

Strain isolation and antimicrobial susceptibility testing is a standard method for the diagnosis of MDR Salmonella spp. (Lin *et al.,* 2000; Malik *et al.,* 2002; Bhutta *et al.,* 2006). Isolation of the organism from blood is the primary diagnostic method, and it takes a minimum of 72 hours for a positive culture report (Kalhan *et al.,* 1998). Approximately around 90% of positive strains can be found during the first week of illness, and this will decrease up to 75% in the second week, 60% in the third week, and 25% in the fourth and subsequent weeks (Wain *et al.,* 1998). Generally Salmonella spp. will appear on the first subculture; if not so it should be repeated to further days until colonies appear. A negative result should only declare after 10-11 days which is considered as complete incubation period (Kalhan *et al.,* 1998); this phenomenon is considered as a drawback and delaying the treatment. Once positive culture was found, the susceptibility tests should be performed with the following antibiotics to confirm MDR: 1) first-line antibiotics such as ampicillin, Chloramphenicol, sulfamethoxazole/ trimethoprim; 2) a fluoroquinolone; 3) nalidixic acid (for determining reduced susceptibility to fluoroquinolone); 4) a third-generation cephalosporin. There are several factors involved in the failure of strain isolation from the blood as (i) insufficient media; (ii) previous use of antibiotics; (iii) insufficient volume of the blood; (iv) blood collection time and incubation time (Lin *et al.,* 2000; Bhutta *et al.,* 2006).

The second method for identification of positive strain is bone marrow aspirate culture having a sensitivity of 80% to 95% (Hawkey, 1998). However bacteria found in the bone marrow is ten times greater than in blood; this is because bacteria may be protected from the presence of circulatory antibiotics (Wain *et al.,* 2008; Farrar *et al.,* 2001). Bone marrow culturing is particularly preferred for patients those who have been previously treated with a long history of illness and for whom has a negative blood culture. The main drawbacks of following bone marrow aspirations for culture purpose include the need for sophisticated instrumentation and managing patient pain (Newell *et al.,* 2010; Wain *et al.,* 2008). Other than these two major methods; stool cultures and urine culturing are also followed, and the sensitivity ranges between 30% to 35% and 7% to 10% respectively. Both stools culturing, urine culturing methods showed positive only after the first week of illness, and this makes its limited use in the very early phase of the illness (Bhutta *et al.,* 1991). Even though the stool culturing method is less important for diagnosis, it shows a significant role in monitoring the carriages of *S.typhi,* after a clinical cure, a carrier may make threats of *Salmonella* to their families (Wain *et al.,* 2008). Widal test is commonly performed and shows positive after the first-week illness (Lin *et al.,* 2000). However the test is inexpensive and easy to perform, it has better sensitivity and specificity with negative results in up to 30% (Bhutta, 2006). Moreover, the Widal test confirms the presence of *Salmonella* spp. And not affords any information regarding antibiotic susceptibility.

Treatment

MDR *Salmonella* spp. Majorly affects children below 10 years and shows more incidence of thelife-threatening syndrome than others (Kumar *et al.,* 2007). Supportive treatment such as administration of antipyretics and maintaining of water level, nutrition in the body may lessen the symptoms (Wain *et al.,* 2008).

In developing countries, antimicrobial agent selection for MDR *Salmonella* is difficult due to it's a cost and less availability. As per WHO guidelines, based on the fluoroquinolone sensitivity third-generation cephalosporin or fluoroquinolone may be preferred for MDR *Salmonella*. Once quinolone-sensitive MDR strains have been found, the best choice is fluoroquinolone for treatment as they have more specificity than third-generation cephalosporins, and also these having advantages like less expensive, availability as an oral preparation, low duration of therapy and high curing rate (Bavdekar, 1996). Ofloxacin and ciprofloxacin also have equal specificity and availability, but norfloxacin is comparatively not preferred because of its poor bioavailability. In recent times gatifloxacin a member of fluoroquinolone also suggested which acts little different from others of the group. Current studies reported many MDR strains of *Salmonella* expressed sensitivity against first-line antibiotics which showed resistant previously since; withdrawal of selective pressure may cause susceptibility to these first-line drugs (Gupta *et al.,* 2009; Manchanda *et al.,* 2006; Prajapati *et al.,* 2008; Wasfy *et al.,* 2002). At final

chloramphenicol and ampicillin are the best choices for wild as well as MDR *Salmonella* because of its less expensive, bioavailability and well proven in-vivo efficiency in developing countries are also the reason for reuse of these antibiotics (Sood *et al.,* 1999).

Prevention and control

Polluted water and foods are the imperative vehicles for *Salmonella* transmission. Historical data proposed that enteric fever was endemic in Western Europe, North America and that rate were decreased after proper treatment of municipal water, pasteurisation of dairy products, and the keeping out of food from faeces contamination (Crump *et al.,* 2010).

Vaccines: At present, there are two vaccines existing for typhoid fever such as Ty21a and parenteral Vi vaccine. Ty21a is a live, attenuated, oral vaccine and the parenteral Vi vaccine is based on the Vi antigen. Ty21a vaccine is available as enteric capsules. Both vaccines are licensed in the United States (Sur *et al.,* 2009). In addition, efforts are underway to develop and evaluate improved live, attenuated, oral vaccines with the goals of maintaining safety while improving efficiency and reducing the number of doses required (Marathe *et al.,* 2012).

Non-vaccine: A century ago, developed countries enlarge better sanitation, accessibility of safe water and food that was achieved but low-and-middle income countries still underway to achieve and this may be the foremost reason for emerging of MDR strains. A recent report suggests that improving the quality of drinking water may be comparatively more vital for the prevention of enteric infection. Although centrally treat water is an important goal, and many research suggests improving the water quality in both household level and at the source. These measures may majorly reduce diarrhoea (Clasen *et al.,* 2007). Anti-Vi antibody assays have proven to be a useful alternative for identifying carriers in outbreak settings (Gupta *et al.,* 2006).

CONCLUSION

Multidrug-resistant *Salmonella* strains which are a causative agent of enteric fever are resistant to the first-line recommended drugs such as chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole. Emerging of MDR may be both natural and sometime non-natural phenomenon; the inappropriate food-handling and poor infection prevention may pave the path to emerge and encourage the further spread of MDR. Since the mid-1980s, MDR *Salmonella* has caused outbreaks in numerous countries in the developing world, resulting in increased morbidity and mortality, especially in children. Bearing the consequences of MDR in mind, this paper accentuates the problems related to MDR and the need to understand its implication and mechanisms to combat microbial infections. Salmonellosis continues to be a major public health difficulty worldwide. It also contributes to downbeat financial impact. The comprehension about MDR *Salmonella* and its evolution is more important to make sure the safety and quality of food. Intervention strategies are hence vital to control *Salmonella* from farm to fork. As such, research on *Salmonella* has gained interest and concern from scientists. There is a need for a strong association between physician and laboratory to choose antibiotics for the treatment of MDR *Salmonella*. Some recent studies show medicinal plant extracts also can act against MDR microbes. Further investigation of these medical plant activities should be encouraged by the government to improve from *in vitro* to *in vivo* level. Government and researchers should concentrate on some advanced level of investigation to suppress or prevent MDR *Salmonella* outbreaks and infections.

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