Original Research Article

LACTOSE FERMENTING *SALMONELLA SPP* IN AKURE: ANTIBIOTIC RESISTANT PATTERNS AND RESULTING CLINICAL IMPLICATIONS.

Abstract

A total of 200 human and non-human samples from Akure metropolis were subjected to bacteriological analysis, of which 37 isolates of lactose fermenting *Salmonella* were obtained; Antibiotic sensitivity tests were carried out on the lactose fermenting isolates of *Salmonella spp* obtained from the samples. From the results, it was discovered that all the lactose fermenting isolates of *Salmonella* showed multiple antibiotic resistances (multidrug resistance) to the different broad spectrum antibiotics used at varying standard inhibitory concentrations. This conducted study gave an insight into the rising incidence of relapsing salmonellosis due to multiple antibiotic resistant strains of lactose fermenting *Salmonella* in the Akure metropolis , provided a scientific explanation to the modified feeding patterns as seen in the *Salmonella* isolates that can utilize lactose sugar due to their genetic modifications and critically evaluated the resulting clinical implications of these residual multiple antibiotic resistant- lactose fermenting *Salmonella* isolates obtained from the metropolis between July and October 2014 during which this research was conducted.

Keywords: Lactose fermenting *Salmonella,* Multiple antibiotic resistances, clinical implications, Enteric relapsing Salmonellosis, Genetic modifications.

Introduction

Salmonellosis is an infectious disease of humans and animals caused by organisms of the genus *Salmonella*. 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; Yousef and Carlstrom, 2003; Montville and

Matthews, 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. 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; Yousef and Carlstrom, 2003; Montville and Matthews, 2008).

Salmonellosis has been recognized in all countries, there are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths worldwide (Pui *et al.* 2008). Salmonellosis can affect all ages, but the incidence and severity of disease is higher in young children, the elderly, and people who are immunocompromised or have debilitating diseases (Boyen *et al.* 2008). Several serovars are host specific (e.g. *S. abortusovis* in sheep or *S. Typhi* in humans) or host adapted (e.g. *S. choleraesuis* in pigs and *S. Dublin* in cattle). Enteric disease is the commonest clinical manifestation, but a wide range of clinical signs, which include acute septicemia, abortion, arthritis and respiratory disease, may be seen. (Newell *et al.*, 2010)

In the United States, approximately 2 to 4 million cases of *Salmonella* gastroenteritis occur with about 500 deaths per year. A more accurate figure of salmonellosis is difficult to determine because normally only large outbreaks are investigated whereas sporadic cases are under-reported. Data on salmonellosis are scarce in many countries of Asia, Africa and South and Central America where only 1 to 10% of cases are reported, (Greene *et al.* 2008). Several outbreaks of Salmonellosis have been reported by Centers for Disease Prevention and Control; these includes the outbreak of the serotypes *enteritidis (PT21and PT1), typhi, tennesse, agona*

and saint-paul respectively between years 2005-2010 which affected not less than 3,000 persons altogether in both United states and England, these outbreaks are implicated to have their sources from contaminated food substance (CDC, 2010; Montville and Matthews, 2008).

Moreover, decades of indiscriminate use and abuse of antibiotics resulted in increased development of antibiotic resistant *Salmonella spp* to different antibiotics, creating a major problem in treatment of salmonellosis and other enteric diseases. There have been many cases of disease relapse and higher incidences in mortality of patients affected by relapsing salmonellosis as mortality rate can be as high as 20%, in the elderly (Hamdan *et al.* 2008). 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). 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).

Salmonella species are generally considered to be unable to ferment lactose and sucrose according to Bergey's Manual. However, Anthony, 1982 and Ewing *et al.* 1986 reported two separate species of *Salmonella* capable of fermenting lactose and sucrose. The organism reported here again illustrates that certain strains of *Salmonella* are capable of fermenting lactose and sucrose rapidly and can resemble very a strain of *Citrobacter freundii*. Greene *et al.* 2008 and Hasan *et al.,* 2009 provided more comprehensive details of a close resemblance, both biochemically and serologically, between *Salmonella tennessee* and *Citrobacter freundii*; the results of which were evidences that certain subspecies or serotypes of *Salmonella* can develop

ability to degrade lactose due to a wide range of environmental or genetic factors such as mutation or natural selection to enable the organism survive outside its nutritional preferences. It has also been described in recent studies that certain lactose fermenting strains of *Salmonella* posses increased antibiotic resistances chiefly because these isolates are already mutated, hence they are genetic variants as when compared to normal bacterial genes encoding for lactose fermentation in other normal *Salmonella* strains (Hasan *et al.,* 2009).

Hence, this study is therefore of great importance in understanding the antibiotic resistant patterns of obtained lactose fermenting *Salmonella spp* in Akure metropolis implicated in the rising incidence of the relapsing enteric fever in the town as this would help to ascertain the carrier rate of the resistant isolates from different sample sources, and offer scientific explanations in relating the gene modifications of these isolates to their possession of multiple antibiotic resistant mechanisms.

MATERIALS AND METHODS

Sample collection

Two hundred samples were collected from both human and non-human sources in Akure between July and October, 2014. Out of these, a total of 80 human stool samples and 40 urine samples were collected from seven different hospitals in all in the metropolis. The samples were collected by Medical Laboratory Scientists in the different hospitals, into labeled sterile universal bottles. A total of 80 water samples were collected into labeled sterile universal bottles from different water sources in different parts of Akure metropolis. All the samples collected were analyzed in the laboratory.

Sample preparation and Standardization of Inoculum

Hamdan *et al.,* 2008 was adopted in which sterile distilled water was used as diluents for the non-human samples, while sterile peptone water was used as diluents for stool and urine samples prior to analysis. A 1ml of each stock was taken using a sterile syringe into 9ml of sterile distilled water or sterile peptone water for serial dilution procedure in sterile test tubes under aseptic conditions until four different dilutions were obtained. Hence, 1 ml of the each dilution factor was used for inoculating the already prepared Nutrient Agar incubated for bacterial isolation at 37^{o}C for 24 hours. After the incubation time, the culture plates were observed for determination of colony forming units and thereafter, the fourth dilution factor was established as the standard for the isolation of the microbes due to easy numerical estimation of the colony forming units on the agar plate of the last dilution factor according to Cheesebrough, 2010, Hamdan *et al.,* 2008.

Biochemical characterization and identification of lactose fermenting *Salmonella*

Olutiola *et al.,* 2001 and Hasan *et al.,* 2009 were adopted by subjecting various obtained sub cultured distinct colonies to a wide array of biochemical tests for characterization and identification. Gram staining technique, Catalase test, Motility test, Sugar fermentation (Glucose and lactose sugars), Citrate test and Acid gas production test were carried out on the distinct isolates obtained after sub culturing. The distinct biochemically characterized colonies were then further sub cultured on Salmonella-Shigella agar and incubated at incubated at 37^{o}C for 24 h. The Citrate test distinguished suspected lactose fermenting isolates of *Salmonella* from isolates of *C. fruendii* in their inability to degrade citrate broth.

Sub-culturing, Characterization and Preservation of Isolates

Distinct colonies on the agar plate were picked aseptically and streaked on Salmonella-Shigella agar, incubated at 37^{o}C for 24 h. Colonies of *Salmonella* spp appear as either pale coloured colony with black centers for H_2S producing *Salmonella* or as Pale coloured colonies without black centers for non H_2S producing strains of *Salmonella* on the Salmonella-Shigella agar (Hamdan *et al.,* 2008; Yoke-Kqueen *et al.,* 2007). Moreover, the suspected *Salmonella* isolates were subjected to various biochemical tests, for identification and characterization (Cheesebrough, 2010). The identified pure isolates of *Salmonella* were preserved on Nutrient Agar Slants and stored at -7^{o}C as described by Cheesebrough, 2010.

Antibiotic Sensitivity Test

The Kirby-Bauer test, also known as disc diffusion method was used to determine the effect of standard antibiotics on bacterial isolates on Mueller Hinton agar. The agar was seeded with 18 hold pure broth cultures of *Salmonella* isolates. The discs were applied unto the seeded plates and incubated for 24 h at 37^{o}C (Cheesebrough, 2010). The bacterial isolates were tested against a wide range of antibiotics namely; Ofloxacillin (5µg), Amoxicillin (25µg), Ciprofloxacin (10µg), Tetracycline (30µg), Pefloxacin (5µg). Thereafter, a ruler was used to measure the diameter of the clear zones of inhibition noticed on the plates and this was noted as degree of antibiotic resistance as described in Zanpantis, 2005. The isolates' zones of inhibition was classified into susceptible (17mm and above), intermediate (13mm-17mm), and resistant (0-12mm) based on the specified standard of mean zone of inhibition for pathogenic gram negative bacilli (Cheesebrough. 2010).

Data analysis

Each treatment was replicated three times. Data obtained were subjected to analysis of variance and treatment means were separated using Duncan's New Multiple Range test at P≤ 0.05 level of significance.

RESULTS

A total of 37 isolates of lactose fermenting *Salmonella spp* were screened out from the 200 samples collected (Table 1); 30 isolates from the human samples were lactose fermenters while only 7 isolates from the non-human samples were discovered to be capable of utilizing lactose in fermentation (Table 2). The isolates' zones of inhibition were classified into susceptible (17mm and above), intermediate (13mm-17mm), and resistant (0-12mm) based on the specified standard of mean zone of inhibition for pathogenic gram negative bacilli (Cheesebrough. 2010; Zanpantis *et al.*2005) and the replicated treatment means were subjected to statistical analysis using Duncan's New Multiple Range test at P≤ 0.05 level of significance as represented in Tables 3a and 3b. Generally, multiple antibiotic resistances was noticed in all the Lactose fermenting isolates of *Salmonella* from the human and non-human samples sources as indicated in Table 4. However, as a matter of reference, adult males and females were discovered to be chronic carriers of the lethal, multiple antibiotic resistant lactose fermenting *Salmonella* isolates amongst the isolates obtained from the human sources, while the river water was the leading reservoir for the lactose fermenting *Salmonella* isolates obtained from the non-human sources.

Table 1: Distribution of Lactose fermenting *Salmonella spp* in human and non-human samples

Sample source	LFSS	NLFSS
RW	4	16
ADAMU	3	1
AWW	3	11
ADAFU	4	7
ADAMS	6	3
AHMCS	3	9
ADAFS	4	8
ADFCS	4	7
ADagMS	3	-
ADagFS	3	-

Keys: LFSS—Lactose fermenting *Salmonella spp*, NLFSS---Non-lactose fermenting *Salmonella spp*, ADAMU—apparently diseased adult female urine ages (18-45yrs), ADAFU—apparently diseased adult female urine ages (18-45yrs), ADAMS—apparently diseased adult male stool ages (18-45yrs), AHMCS--- apparently healthy male children stool ages (6-17 yrs), ADFCS—apparently diseased female children stool ages (6-17 yrs), ADAFS—apparently diseased adult female stool ages (18-45yrs), ADagMS—apparently diseased aged male stool ages (55-80 yrs), ADagFS--- apparently diseased aged female stool ages (55-80 yrs), RW- River water samples, AWW- Abattoir waste water samples.

174 Table 2: Biochemical characteristics of lactose fermenting *Salmonella* spp isolated from non-human and human samples

Code of isolates	Gram stain	Catalase	Motility test	Glucose fermentation	Lactose fermentation	Citrate test	Colony morphology	Colour on SSA	Suspected Organism	Number of isolates
LFSSNHS	-ve (bacilli rods)	+ve	+ve	Acid /gas	Acid /gas	+ve	Cream/ raised	PCC+BC	*Salmonella spp*	7
LFSSHS	-ve (bacilli rods)	+ve	+ve	Acid /gas	Acid /gas	+ve	Cream/ raised	PCC+BC	*Salmonella spp*	30

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176 Keys: LFSSNHS—Lactose fermenting *Salmonella spp* in non-human samples, LFSSHS-- Lactose fermenting *Salmonella spp* in human samples,-ve—Negative,

177 +ve—Positive, SSA—Salmonella-Shigella agar PCC+BC—Pale coloured colonies with black centers (indicating lactose fermenting isolates and H_2S producers).

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Table 3a: Zones of inhibition of Lactose fermenting *Salmonella* isolates in human and non-human samples

Codes of isolates	OFL	AMX	CPX	TET	PFX
SSADAMU	14.33±2.52^{b}	10.67±2.51^{c}	16.00±2.00^{b}	00.00±2.65^{c}	21.00±3.00^{b}
	14.33±2.52^{b}	8.67±1.53^{b}	16.33±2.52^{b}	00.00±2.00^{b}	21.00±3.00^{b}
	14.33±2.52^{b}	00±00^{a}	15.33±1.53^{b}	00.00±1.00^{b}	20.00±2.00^{b}
SSAHMCS	17.67±2.52^{b}	10.00±2.00^{b}	18.00±2.00^{b}	11.67±1.15^{b}	14.67±2.52^{b}
	17.00±2.00^{b}	00±00^{a}	17.67±1.53^{b}	00±00^{a}	15.67±2.52^{b}
	19.00±1.00^{c}	00±00^{a}	19.00±1.00^{b}	00±00^{a}	19.33±2.52^{c}
SSADAFU	15.00±1.00^{b}	00±00^{a}	17.00±1.00^{b}	00±00^{a}	13.00±2.00^{b}
	18.00±1.00^{d}	00±00^{a}	18.00±1.00^{c}	00±00^{a}	16.00±2.00^{c}
	17.00±1.00^{c}	00±00^{a}	19.00±1.00^{d}	00±00^{a}	00±00^{a}
	12.00±1.00^{c}	00±00^{a}	12.00±1.00^{a}	6.00±00^{b}	00±00^{a}
SSADAMS	14.00±2.00^{b}	9.00±2.00^{b}	16.00±2.00^{b}	8.00±2.00^{b}	8.67±2.52^{b}
	16.67±1.53^{c}	00±00^{a}	00±00^{a}	00±00^{a}	14.00±2.00^{c}
	00±00^{a}	00±00^{a}	00±00^{a}	00±00^{a}	00±00^{a}
	00±00^{a}	00±00^{a}	00±00^{a}	00±00^{a}	00±00^{a}
	00±00^{a}	00±00^{a}	00±00^{a}	18.00±2.00^{b}	10.00±2.00^{b}
	00±00^{a}	00±00^{a}	00±00^{a}	18.00±2.00^{b}	00±00^{a}
SSADAFS	17.00±1.00^{b}	00±00^{a}	15.67±1.53^{b}	16.00±2.00^{b}	15.00±2.00^{b}
	19.00±1.00^{c}	00±00^{a}	16.67±1.53^{b}	00±00^{a}	15.00±2.00^{b}
	13.50±1.00^{a}	5.45±1.00^{a}	11.00±1.00^{a}	12.10±1.00^{b}	00±00^{a}
	00±00^{a}	00±00^{a}	11.42±1.20^{a}	10.50±1.24^{b}	00±00^{a}
SSAWW	20.67±2.52^{b}	6.33±2.51^{b}	17.67±2.04^{b}	13.00±2.00^{b}	17.67±2.52^{b}
	23.33±2.52^{b}	5.67±2.51^{b}	16.00±2.00^{b}	13.00±2.00^{b}	17.67±2.52^{b}
	24.00±2.00^{b}	5.00±2.00^{b}	17.00±2.00^{b}	12.00±1.00^{b}	17.33±1.57^{b}

Key: SSADAMU-- *Salmonella spp* from apparently diseased adult male urine (ages 18-45 yrs), SSAHMCS---*Salmonella spp* from apparently healthy male children stool (ages 6-17 yrs), SSADAFU---*Salmonella spp* from apparently diseased adult female urine (ages 18-45 yrs), SSADAMS—*Salmonella spp* from apparently diseased adult male stool ages (18-45yrs), -(SSADAFS)—*Salmonella spp* from apparently diseased adult female stool ages (18-45yrs), SSAWW--- *Salmonella spp* from Abattoir waste water samples, OFL---Ofloxacillin, AMX----Amoxycillin, CPX---Ciprofloxacin, TET---Tetracycline, PFX---Pefloxacin, Values with the same letter as superscript have no significant difference at $p \leq 0.05$ level of significance.

Table 3b: Zones of inhibition of Lactose fermenting *Salmonella* isolates in human and non-human samples

Codes of isolates	OFL	AMX	CPX	TET	PFX
SSADagFS	19.00±1.00^{b}	13.00±2.00^{b}	17.00±2.00^{b}	00±00^{a}	18.00±2.00^{b}
	21.67±2.52^{b}	14.67±1.53^{b}	15.00±2.00^{b}	12.00±2.00^{b}	18.67±1.53^{c}
	23.00±2.00^{c}	00±00^{a}	16.00±2.00^{b}	11.67±1.53^{b}	17.00±1.00^{b}
SSADagMS	14.67±2.52^{b}	10.00±2.00^{b}	18.00±2.00^{b}	11.67±1.15^{b}	17.67±2.52^{b}
	15.00±2.00^{b}	00±00^{a}	17.67±1.53^{b}	00±00^{a}	15.67±2.52^{b}
	15.00±1.00^{c}	00±00^{a}	19.00±1.00^{b}	00±00^{a}	19.33±2.52^{c}
SSADFCS	15.00±1.00^{b}	00±00^{a}	17.00±1.00^{b}	00±00^{a}	13.00±2.00^{b}
	18.00±1.00^{d}	00±00^{a}	18.00±1.00^{c}	00±00^{a}	16.00±2.00^{c}
	17.00±1.00^{c}	00±00^{a}	19.00±1.00^{d}	00±00^{a}	00±00^{a}
	13.00±1.00^{a}	00±00^{a}	15.00±1.50^{b}	00±00^{a}	12.05±1.00^{b}
SSRW	15.00±2.00^{b}	19.00±2.00^{b}	16.67±1.53^{b}	13.00±2.00^{b}	18.00±2.00^{c}
	16.67±1.53^{b}	18.00±2.00^{b}	19.67±1.53^{c}	13.70±2.00^{b}	19.00±2.00^{c}
	5.33±1.50^{a}	00±00^{a}	16.33±2.52^{b}	8.00±1.00^{a}	00±00^{a}
	00.00±0.00^{a}	00±00^{a}	21.33±2.52^{c}	6.00±1.00^{a}	00±00^{a}

Keys: SSRW--- *Salmonella spp* from River water samples, SSADagMS-- *Salmonella spp* from apparently diseased aged male stool (ages 55-80 yrs), SSADagFS-- *Salmonella spp* from apparently diseased aged female stool (ages 55-80 yrs), SSADFCS---*Salmonella spp* from apparently diseased female children stool (ages 6-17 yrs), OFL---Ofloxacillin, AMX----Amoxycillin, CPX---Ciprofloxacin, TET---Tetracycline, PFX---Pefloxacin. Values with the same letter as superscript have no significant difference at $p \leq 0.05$ level of significance.

Table 4: The deduced antibiotic resistance patterns of the Lactose fermenting *Salmonella* isolates from non-human and human samples after estimation of their inhibition zones

Codes of isolates	Number of isolates	OFL	AMX	CPX	TET	PFX	INFERENCE
SSADAMU	3	3I	3R	3I	3R	3S	MARI
SSADAFU	4	2S, 1I, 1R	4R	R, 3S	4R	2I, 2R	MARI
SSADAMS	6	4R, 2I	6R	1I, 5R	2S, 4R	1I, 5R	MARI
SSAHMCS	3	3S	3R	3S	3R	3I,1S	MARI
SSADAFS	4	2S, 1I, 1R	4R	2I, 2R	1I ,3R	2I, 2R	MARI
SSADFCS	4	4I	4R	4S	4R	1S, 2I, R	MARI
SSAWW	3	3S	3R	2S, 1I	2I, R	3S	MARI
SSRW	4	2R, 2I	2S, 2R	2I, 2S	2I, 2R	2S, 2R	MARI
SSADagMS	3	3I	3R	3S	3R	2I, 1S	MARI
SSADagFS	3	3S	1R, 2I	3I	3R	3S	MARI

Key: SSADAMU-- *Salmonella spp* from apparently diseased adult male urine (ages 18-45 yrs), SSADAFU---*Salmonella spp* from apparently diseased adult female urine (ages 18-45 yrs), SSADAMS--*Salmonella spp* from apparently diseased adult male stool (ages 18-45 yrs), SSADAFS---*Salmonella spp* from apparently diseased adult female stool (ages 18-45 yrs), SSAHMCS---*Salmonella spp* from apparently healthy male children stool (ages 6-17 yrs), SSADFCS---*Salmonella spp* from apparently diseased female children stool (ages 6-17 yrs, SSADagMS--*Salmonella spp* from apparently diseased aged male stool (ages 55-80 yrs, SSADagFS--*Salmonella spp* from apparently diseased aged female stool (ages 55-80 yrs), SSRW--- *Salmonella spp* from River water samples, SSAWW--- *Salmonella spp* from Abattoir waste water samples, MARI—Multiple antibiotic resistant isolates, R—resistant, S—susceptible, I—intermediate, OFL---Ofloxacillin, AMX----Amoxycillin, CPX---Ciprofloxacin, TET---Tetracycline, PFX---Pefloxacin.

DISCUSSION

It was discovered from this study that, more lactose fermenting isolates are resident in human sources than in non-human sources; the high occurrence of the lactose fermenting *Salmonella* in human samples is possibly due to a couple of factors including mutation and increased exposure of human carriers of *Salmonella* isolates to lactose containing substrates of dairy products, this resulting in nutritional adaptation of the isolates of *Salmonella spp* to the nutritional environment and hence ability to utilize and ferment lactose this agrees with the findings of Anthony, 1982; Hamdan *et al.,* 2008; Greene *et al.* 2008 and Hasan *et al*, 2009.

Subsequently, as a result of mutagenic variations in the lactose fermenting *Salmonella* isolates; multiple antibiotic resistances were noticed in all the isolates ranging from each class of the human and non –human samples. The multiple antibiotic resistances observed is due to a variety of factors which includes indiscriminate use of drugs by human sample donors, faecal contamination of different water sources by human carriers of lactose fermenting *Salmonella spp* where samples were collected from the metropolis resulting in the introduction of drug resistant isolates of *Salmonella* to different water bodies. This agrees with similar studies conducted in other parts of the world as described in the findings of Hamdan *et al.,* 2008; Yoke-Kqueen *et al.,* 2007. Thus, the spread of the multiple antibiotic resistant-lactose fermenting *Salmonella spp* was also implicated in a study carried out by *Zhao et al.,* 2003 and Greene *et al.* 2008.

Moreso, the multiple antibiotic resistances observed in all the lactose fermenting *Salmonella* isolates obtained from the human sample sources gave an insight into reasons for frequent relapses of salmonellosis amongst patients diagnosed across various health organizations in the Akure metroplolis between July and October, 2014 during which this research study was

conducted. Hence, adequate medical laboratory tests should be carried out on patients with suspected cases of enteric fever or salmonellosis before treatments are recommended to them; this would help curb the development and spread of antibiotic resistance in many serovars of *Salmonella* in the Akure metropolis. Different antibiotic combination therapies are also strongly recommended for prophylactic treatment of infected patients.

CONCLUSION

It is recommended that public health awareness be given to commercial artisans and patients about the spread of enteric diseases in Akure metropolis, better epidemiological surveillance can also be mounted on food vending joints, major water sources and hospitals to reduce the incidence of relapsing salmonellosis caused by multiple antibiotic resistant lactose fermenting *Salmonella* isolates. Herd immunity of infants, aged and pregnant women and immunocompromised individuals can also be implemented in primary health care centers in the metropolis.

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