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Potential control of *Salmonella* spp. isolated from different environmental sources by combined mixture of henna, garlic and onion extracts

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ABSTRACT

All *Salmonella* serotypes showed a great diversity in their resistance patterns. The enormous expansion of resistance of *Salmonella* to certain antimicrobials is particularly causing problems, therefore, this study aimed to investigate a new natural compound to inhibit *Salmonella* serotypes. One hundred and twenty bacterial isolates were recovered from different foods and environmental samples. All isolated *Salmonella* were resistant to cefaclor with percentage of 100%, and were 100% susceptible to ciprofloxacin, norfloxacin, ertapenem and nitrofurantoin. Fourteen multidrug resistant (MDR) *Salmonella* serotypes were controlled by different plant extracts especially a combination mixture of henna plant, fresh garlic and onions extracts. These MDR isolates were examined for the existence of 6 virulence genes (*inv*A, *stn, ssa*Q, *gip*A, *sop*B and *spv*C) and their relationship with antibiotic resistance. No virulence genes; *gip*A and *ssa*Q genes were detected in the tested *Salmonella* serotypes, whereas possess *inv*A, *stn* and *sop*B genes. Overall, the most resistant virulent isolated *Salmonella* serotypes were *S.* Vejle, *S.* Meleagridis and *S.* Anatum which have four virulence genes (*inv*A, *stn, sop*B and *spv*C). The consequences of this investigation will help in grasping the spread of virulence genotypes and antibiotic resistance in *Salmonella*. The prospectivity of adding henna active ingredients besides garlic and onion as mixtures may be potential for *Salmonella* infectious diseases.

1. Introduction

Salmonella is rod-shaped, flagellated facultative anaerobes (Montville and Matthews, 2008). With a complicated terminology, it involves two species, where, there are six subspecies with more than 1500 serotypes in *S. enetrica* only. *S. enterica* subsp. *enterica* is major food pathogen and accountable for over 99% of human salmonellosis so it is extensively speculated (Lamas et al., 2018), with 2610 different serotypes (Su and Chiu, 2007). Most warm-blooded animals concealed *S. enterica* in their gut (Kalita et al., 2014), causing intestinal diseases in humans and animals worldwide (Rabsch et al., 2001). The infection dosage brings off 10^5 cells causing typhoid fever, whereas 10^3 organisms can't enhance clinical ailments (WHO, 2011). Gastroenteritis, enteric (typhoid) fever, and non-typhoidal salmonellosis diseases with bacteremia are considered as the three main types of diseases caused by *Salmonella* strains in people. Each clinical disorder demands specific sets of genes where, hereditary examination demonstrates that their pleiad of virulence highlights is extraordinarily contrast (Guiney and Fierer, 2011). Sustenance flare-ups from this pathogen haven't declined by Centers for Disease Control and Prevention (Chen et al., 2017). Gastroenteritis, likewise food poisoning, is all the more normally brought about by the non-typhoidal serotype Typhimurium. Numerous clinical signs describes the infection with 8–72h after ingestion, for example, sickness, regurgitating, stomach issues, looseness of the bowels, and migraine. Complications can lead to systemic infections and various chronic conditions (Montville and Matthews, 2005). Bacteremia happens for the most part in immunocompromised patients and whose with HIV-AIDS, diabetes, sickle cell malady. Bacteremia can cause septic shock; endocarditis, disease of the aorta, urinary tract contamination, pneumonia; pulmonary abscess (Bronze and Greenfield, 2005; Ryan and Ray, 2004).

The gene analysis of *S. enterica* showed particular sets of genes that are existing in pathogenicity islands creating diverse characteristic clinical syndromes of typhoid fever, enteritis, or non-typhoid infection (Fierer and Guiney, 2001). *Salmonella* encodes two T3SSs, which are needed for various steps of salmonellosis (Lamas et al., 2018). Adhesion, invasion, and toxin genes are grouped in SPI (Santos et al., 2003). Repeating units are flanking the SPI with varied G/C content (Schmidt

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and Hensel, 2004).

More than 20 SPIs have been described where, the large gene cassettes within the *Salmonella* chromosome harbor diverse genes such as *inv*A, *spv*, *fim*A and *stn* which are required for salmonellosis (Sabbagh et al., 2010). The *inv*A gene codes for a protein required for invasion of epithelial cells (Darwin and Miller, 1999). Whereas, an important-plasmid operon (*spv*RABCD), contains five genes, that is commonly associated with some serotypes. the *spv* operon is responsible for potentiating *Salmonella* dissemination (Heithoff et al., 2008). While, the *spv*C gene is responsible for survival within host cell (Chiu and Ou, 1996). The *stn* gene also is responsible for production of enterotoxin which induce gastroenteritis diseases (Chopra et al., 1987).

So, the great adaptation of *Salmonella* may be due to their genetic texture. As well as, the MDR *Salmonella* strains emanation poses a great challenge of effective treatment. The limitation of random use of antibiotics in food animals is one of the most effective systems to prevent *Salmonella* dissemination (Eng et al., 2015). Until two decades ago, reported *Salmonella* outbreaks predominantly involved chicken, turkey, pork, meat, and eggs (CDC, 2016).

Multidrug-resistant *S.* Typhi and non-typhoidal *Salmonella* (NTS) are increased and are often associated with HIV infection (Ruby et al., 2012). It was suggested that universal antibiotic exhaustion increased according to national pharmaceutical sales (Van Boeckel et al., 2014). This attributed elevation is due to preferring cephalosporins for enteric fever among others resulting in rising antimicrobial resistance (AMR) for other antimicrobials (Klein et al., 2018). Three classes of antibiotics were most consumed, beta-lactams, macrolides, and fluoroquinolones. MDR *S.* Typhi in Asia was declined, whereas resistance to nalidixic acid and fluoroquinolones continued to increase. Third-generation cephalosporin resistance rose while, Azithromycin may be used (Britto et al., 2018).

Most antibiotics had a neutral or negative effect on motility. The carefully use of antibiotics is critical due to the effect of antibiotic on MDR *Salmonella* motility and to minimize its consequences (Brunelle et al., 2017).

So, due to the bacterium's high bearing to environmental harsh conditions, prevalence distribution, multiple drug resistance, and modification, there are many impediments for its control (Chen et al., 2013). Therefore, Plant extracts can be used as natural preservatives for some foods (Kim et al., 2004). The suitable antimicrobial handling depends on laboratory sensitivity test in rich countries, but this is not probable in poor societies (Szych et al., 2014). Henna leaves have antimicrobial activity rather than the seeds, with limited efficiency and at higher concentrations. the leaves showed some activity against *Candida albicans* but the seeds not (Fessenden and Fessenden, 1998). The oxidation-reduction reactions of diphenol and diketone gives its dyeing property and play a vital role in biological systems (Fessenden and Fessenden, 1998). Plants contain a wide variety of secondary metabolites polyphenols with antimicrobial activities, where 50% of current pharmaceuticals are plant in origin (González-Lamothe et al., 2009). Molecules of alkaloids, glycosides, terpenoids, saponins, steroids, flavonoids, tannins, quinones and coumarins can be produced by plants (Das et al., 2010). Such these some natural extracts are efficient in the curing of microbial infections (Fernebro, 2011). Some of these extracts exert a good potential against Gram-negative and Gram-positive bacteria regardless the differences in cell wall structure. The potency of these extracts was not due to the presence of metabolic toxin but possibly through another mechanism of action (Elisha et al., 2017). Rising antibiotic resistance and the scarcity of new antimicrobials has long been acknowledged (Walsh and Toleman, 2012). The great global challenge is the necessity of efficient novel antimicrobial agents to treat such infectious diseases, especially in poor countries around the world, where one-half of deaths are due to infectious diseases (Srivastava et al., 2013).

This work aimed to control the growth of *Salmonella* serovars, isolated from different environmental sources, using cheapest antimicrobial agents including various plant extracts. It aimed also to confirm identification of isolated *Salmonella* by molecular analysis of virulence genes.

2. Material and methods

2.1. Sample collection

A total of 96 environmental samples including tomatoes, acquired from encompassing fields or obtained at roadside produce fruits, luncheons meat, skimmed milk, intestinal of chickens, feces (healthy and diseased animals, eggs, sediment soils and water lake of Burullus(14 water samples intervals) were collected from different governorates of Egypt. The samples were collected at the periods from December 2016 to February 2017. All samples were kept at 4 °C before and during transferring to the laboratory until microbiological analyses.

2.2. Isolation, identification and serotyping of Salmonella

2.2.1. Pre-enrichment in nonselective liquid broth

All samples were pre-enriched overnight at 35 °C for isolation of ***Salmonella*** in modified buffered peptone water consists of (g/L): 10 peptone (Oxoid, England), 5 sodium chloride, 7 disodium phosphate, 3 potassium phosphate (Honeywell, Germany). Tomato natural products were quartered and after that 200 g were set in sterile Whirlpak channel packs (Nasco, Fort Atkinson, WI) and hand squashed to completely blend (Andrews et al., 2011).

Water samples were filtered through 0.45 μm filters. Refineries were then set in Whirlpak filter sacks with 100 ml of pre-enrichment broth. Pond and lake water were concentrated to 250 ml by nonstop stream centrifugation (Scientific Methods, Inc., Granger, IN). Twenty-five ml of the concentrated sample were added to 225 ml pre-enrichment stock in a Whirlpak filter sack. The method for detection, isolation and identification of *Salmonella* was done according to ISO 6579 (2002).

2.2.2. Enrichment in selective liquid media

Aliquot of 0.1 mL of the pre-enrichment cultures was transferred to a tube containing 10 mL of the Rapport Vassiliadis Soy broth, then incubated at 41.5 °C±1 °C for 24h. Also, 1 ml of the pre-enrichment culture was transferred to a tube containing 9 ml of the Muller–Kauffmann tetrathionate/novobiocin (Oxoid, UK) broth, then incubated at 37 °C±1°Cfor 24h.

2.2.3. Plating on solid selective media

Ten μL of the previously inoculated and incubated selective enrichment culture were inoculated onto the surface of Salmonella Shigella agar (SSA)(straw color with black centers), Xylose Lysine Desoxycholate (XLD) (red colonies with black center) and Bismuth Sulphite agar (BSA) (black colonies with metallic sheen) and Hektoen Enteric agar (HE) (green colonies with black center), then incubated at 37 °C±1 °C for 24h.

2.3. Identification of Sallmonella serotypes

Preliminarily identification of *Salmonella* serotypes was performed by studying their biochemically characteristics according to ISO 6579 (2002). *Salmonella* identification was confirmed again by subculture onto MacConkey agar, SS agar and Xylose Lysine Desoxycholate agar (XLD) for several times and by series of biochemical tests (Murray et al., 2003).

2.4. Serotyping of Salmonella

The isolates that were distinguished biochemically as *Salmonella* will be exposed to serological recognizable proof as per Kauffman-White Scheme (Kauffman, 1974) for assurance of somatic (O) and flagellar (H) antigens **(SIFIN and Denka Seiken; Co. Ltd) (ISO 6579–3:2014)** as per the maker's guidelines (Danielsson and Kronvall, 1974**).**

Table 1
Oligonucleotide primers sequences and accession numbers of selected genes.

Target gene	Primers sequences (5–3)	Amplified segment (bp)	Accession number of gene
stn	**F-**TTG TGT CGC TAT CAC TGG CAA CC **R-** ATT CGT AAC CCG CTC TCG TCC	617	- SV (MK127933) - SM (MK127934) - SA (MK127935)
sopB	**F-**TCA GAA GRC GTC TAA CCA CTC **R-** TAC CGT CCT CAT GCA CAC TC	517	- SV (MK127927), - SM (MK127928) SA (MK127929)
spvC	**F-**ACC AGA GAC ATT GCC TTC C **R-** TTC TGA TCG CCG CTA TTC G	467	- SV (MK127930) - SM (MK127931) - SA (MK127932)
ssaQ	**F-**GAA TAG CGA ATG AAG AGC GTC GTC C **R-** CAT CGT GTT ATC CTC TGT CAG C	455	
gipA	**F-** GAA TAG CGA ATG AAG AGC GTC C **R-** CAT CGT GTT ATC CTC TGT CAG C	518	
invA	**F-** GTGAAATTATCGCCACGTTCGGGCAA **R-** TCATCGCACCGTCAAAGGAACC	284	- SV (MK127924) - SM (MK127925) - SA (MK127926)

SV: *Salmonella* Vejle Eg77*,* SM: *Salmonella* Meleagridis BrL115, SA*: Salmonella* Anatum Alb118.

2.5. Antibiotic susceptibility test

The susceptibility of all *Salmonella* serotypes to various antibiotics (Oxoid, Basingstoke, Hampshire, England, UK) was examined by the Kirby-Bauer standard agar disk diffusion technique (Bauer et al., 1966). The tested antibiotics and their concentrations in μg/disk were as the following: **ETP$_{10}$μ$_g$** (ertapenem), **F$_{300}$**μ$_g$ (nitrofurantoin), **OB$_5$μ$_g$** (cloxacillin), **RD$_5$μ$_g$** (rifampcin), **MEM$_{10}$**μ$_g$ (meropenem), **AML$_{10}$**μ$_g$ (amoxycillin), **SXT$_{25}$**μ$_g$ (sulfamethoxazole–trimethoprim), **NV$_5$**μ$_g$ (novobiocin), **CIP$_5$**μ$_g$ (ciprofloxacin), **NOR$_{10}$**μ$_g$ (norfloxacin), **MTZ$_5$**μ$_g$ (metronidazole), **TEC$_{30}$**μ$_g$ (tecoplanin), **B$_{10}$**μ$_g$ (bacitracin), **CEC$_{30}$**μ$_g$ (cefaclor). The repression zones(mm) were estimated and scored as sensitive, intermediate, and resistant classes as per the basic breakpoints prescribed by

Table 2
Susceptibility of *Salmonella* isolates to different antibiotics.

Percentage (%) of resistance of bacterial isolates	Percentage (%) of susceptibility of bacterial isolates	No. of sensitive bacterial isolates	Antibiotic type
0.0%	100%	120	Ertapenem (ETP$_{10}$ μ$_g$)
0.0%	100%	120	NitroFurantoin (F$_{300}$ μ$_g$)
82.5%	17.5%	21	Cloxacillin (OB$_5$ μ$_g$)
50%	50%	60	Rifampcin (RD$_5$ μ$_g$)
18.3%	81.66%	98	Meropenem (MEM$_{10}$ μ$_g$)
20%	80%	96	Amoxycillin (AML$_{10}$ μ$_g$)
13.33%	86.66%	104	Sulfametoxazole –Trimethoprime (SXT$_{25}$)
56.66%	43.33%	52	Novobiocin (NV$_5$ μ$_g$)
0.0%	100%	120	Ciprofloxacin (CIP$_5$ μ$_g$)
0.0%	100%	120	Norfloxacin (NOR$_{10}$ μ$_g$)
85%	15%	18	Metronidazole (MTZ$_5$ μ$_g$)
35.83%	64.17%	77	Tecoplanin (TEC$_{30}$ μ$_g$)
78.33%	21.67	26	Bacitracin (B$_{10}$ μ$_g$)
100%	0.0%	0	Cefoclore (CEC$_{30}$ μ$_g$)

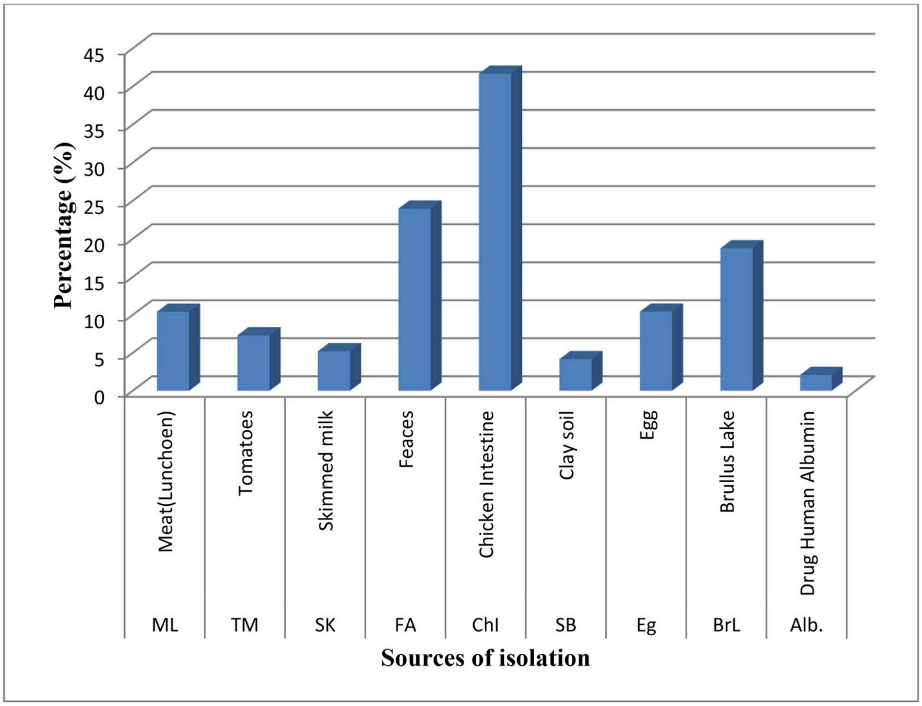


Fig. 1. Distribution of *Salmonella* isolates according to different sources of isolation.

Table 3
Effect of different plant extracts against multidrug resistant and virulent pathogenic Salmonellae strains.

Plant family	Genus and species	Part used	Solvent used	Inhibition zone diameter (mm)			
				F24	ChI51	Eg77	ChI87
Amaryllidaceae	*Allium cepa* (Onion)	bulb	A	0.0	13 ± 0.34c	0.0	12 ± 0.32c
			B	0.0	9 ± 0.24g	0.0	9 ± 0.24e
			C	0.0	9 ± 0.24g	0.0	0.0
	Allium sativum (Garlic)	seed	A	0.0	14 ± 0.37b	0.0	12 ± 0.31c
			B	0.0	8 ± 0.21h	0.0	0.0
			C	0.0	9 ± 0.24g	0.0	0.0
Moringaceae	*Moringa oleifera* (drumstick tree)	seed	A	0.0	11 ± 0.29e	0.0	0.0
			B	0.0	9 ± 0.24g	0.0	0.0
			C	0.0	7 ± 0.18i	0.0	0.0
		leaves	A	0.0	12 ± 0.31d	0.0	0.0
			B	0.0	9 ± 0.24g	0.0	0.0
			C	0.0	0.0	0.0	0.0
Fabaceae	*Albizialebbeck* (flea tree)	leaves	A	11 ± 0.29d	0.0	0.0	9 ± 0.24e
			B	10 ± 0.26e	0.0	0.0	9 ± 0.24e
			C	10 ± 0.26e	0.0	0.0	9 ± 0.24e
		stem	A	10 ± 0.26e	0.0	0.0	10 ± 0.26d
			B	9 ± 0.23f	0.0	0.0	8 ± 0.21f
			C	9 ± 0.23f	0.0	0.0	9 ± 0.24e
Euphorbiaceae	*Euphorpia tirucalli* (naked lady, pencil tree)	stems	A	0.0	0.0	0.0	9 ± 0.24e
			B	0.0	0.0	0.0	8 ± 0.21f
			C	0.0	0.0	0.0	8 ± 0.21f
Urticaceae	*Urtica dioica* (Stinging Nettle)	leaves	A	0.0	0.0	0.0	10 ± 0.26d
			B	0.0	0.0	0.0	9 ± 0.24e
			C	0.0	0.0	0.0	10 ± 0.26d
Lythraceae	*Lawsonia inermis* (Henna)	leaves	A	17 ± 0.45a	17 ± 0.45a	0.0	18 ± 0.49a
			B	15 ± 0.39b	12 ± 0.31d	0.0	15 ± 0.39b
			C	10 ± 0.26e	10 ± 0.26f	0.0	0.0
Purtolacaceae	*Portulaca oleracea* (red root, or parsley)	leaves	A	0.0	11 ± 0.29e	0.0	10 ± 0.26d
			B	0.0	10 ± 0.26f	0.0	10 ± 0.26d
			C	0.0	10 ± 0.26f	0.0	9 ± 0.24e
Lamiaceae	*Ocimum basilicum* (sweet basil)	leaves	A	0.0	0.0	0.0	0.0
			B	0.0	0.0	0.0	0.0
			C	0.0	0.0	0.0	0.0
Asteraceae	*Pluchae discordis* (camphorweeds or Plucheas)	Leaves and flowers	A	9 ± 0.24f	10 ± 0.26f	0.0	7 ± 0.18g
			B	8 ± 0.21	8 ± 0.21h	0.0	7 ± 0.18g
			C	8 ± 0.21g	7 ± 0.18i	0.0	7 ± 0.18g
Rhamnaceae	*Rhamnus cathartica* (purging buckthorn)	leaves	A	10 ± 0.26e	0.0	0.0	8 ± 0.21f
			B	9 ± 0.24f	0.0	0.0	8 ± 0.21f
			C	7 ± 0.18h	0.0	0.0	8 ± 0.21f
Ranunculaceae	*Actaea racemosa* (Black COHOSH)	Fresh root and flower	Ethanol 94%	0.0	10 ± 0.26f	8 ± 0.22a	-ve
Asteraceae	*Echinacea purpura* (hedgehog coneflower)	Echinacea extract	A	10 ± 0.26e	-ve	-ve	-ve
Amaryllidaceae	Allicin extract (Garlic active compound)	active compound	Methainol:Water (3:1)	12 ± 0.32c	9 ± 0.34g	-ve	10 ± 0.26d

A: Methanol. B: Methylene Chloride. C: Boiling water.

the Clinical and Laboratory Standards Institute (CLSI, 2012).

2.6. Molecular Analysis

2.6.1. Detection of virulence genes in selected Salmonella serotypes

Fourteen *Salmonella* serotypes were selected for detection of virulence genes. Six pairs of primers specific for virulence genes; *stn*, *inv*A, *ssa*Q, *gip*A, *sop*B and *spv*C were tabulated in Table 1. QIAamp DNA mini kit instructions, was used in PCR analysis. DNA templates of the tested bacterial cultures were set up from 24-h cultures in tryptone soya broth (TSB) at 30 °C, according to the method described by Kumar et al. (2008)**.** Specific multiplex PCR amplification conditions for *Salmonella* spp. virulence genes were according to Kumar et al. (2008), using two pairs of primers (forward and or reverse) to give product sizes range from 100bp to 617bp using Emerald Amp GT PCR mastermix (Takara). The master mix PCR amplification was performed in a final volume of 20 μl containing 5 μl of DNA template in a final concentration 1 × PCR buffer (10mMTris–HCl, 2,000 μM of each dNTP, 5 U Taq DNA polymerase, 1μ primers. The amplification consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 45 s, annealing at 59, 55 °C and 58 °C for 1 min for *stn, inv*A and (*ssa*Q, *gip*A, *spv*C, *sop*B) genes respectively, and elongation at 72 °C for 45 s for the all genes except *inv*A for 30 s, with a final extension at 72 °C for 7 min. Amplicons were separated in a 1.5% agarose gel and sizes were estimated using Gel Pilot 100 bp ladder QIAGEN (USA) run on the same gel with gene ruler 100 bp DNA ladder Fermentas (Germany).

2.6.2. Molecular characterization of virulence genes of Salmonella serotypes

A purified PCR product were sequenced in the forward or reverse direction in a Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA), using a ready reaction Bigdye Terminator V3.1 cycle sequencing kit (PerkinElmer/Applied Biosystems, Foster City, CA) T3 Thermal cycler (Biometra). The virulence genes sequences which have been determined in the present study were deposited at the NCBI web server (www.ncbi.nlm.nih.gov). A BLAST® analysis (Basic Local Alignment Search Tool) was initially performed to establish sequence identity to GenBank accessions (Altschul et al., 1990). Primers used in this study were synthesized by Metabion International (Germany).

2.7. Phylogenetic analysis

A comparative analysis of sequences was performed using the CLUSTAL W multiple chain sequence alignment program, version 1.83 of MegAlign module of Lasergene DNAStar software Pairwise. Phylogenetic analyses were accomplished acquisition extreme probability,

Inhibition zone diameter (mm)									
Eg103	BrL104	BrL106	BrL108	BrL112	Eg113	BrL115	BrL116	Alb. 118	BrL120
14 ± 0.37c	12 ± 0.32c	13 ± 0.34d	12 ± 0.32d	12 ± 0.32c	13 ± 0.34c	11 ± 0.29b	15 ± 0.69b	12 ± 0.32b	13 ± 0.34c
10 ± 0.26f	8 ± 0.21f	8 ± 0.21h	9 ± 0.24ef	9 ± 0.24f	9 ± 0.24f	8 ± 0.21e	10 ± 0.26e	9 ± 0.24e	9 ± 0.24g
10 ± 0.26f	8 ± 0.22f	10 ± 0.26f	9 ± 0.25ef	9 ± 0.24f	9 ± 0.24f	9 ± 0.24d	8 ± 0.21g	7 ± 0.19g	10 ± 0.26f
15 ± 0.39b	13 ± 0.34b	14 ± 0.37c	14 ± 0.37c	14 ± 0.37 b	13 ± 0.34c	10 ± 0.26c	13 ± 0.34c	11 ± 0.29c	14 ± 0.37b
9 ± 0.24g	10 ± 0.26d	10 ± 0.26f	11 ± 0.29d	11 ± 0.29d	10 ± 0.26e	9 ± 0.24d	10 ± 0.26e	10 ± 0.26d	10 ± 0.26f
10 ± 0.26f	8 ± 0.21f	9 ± 0.24g	8 ± 0.21 fg	8 ± 0.21g	10 ± 0.26e	10 ± 0.26c	8 ± 0.21g	8 ± 0.21f	9 ± 0.24g
11 ± 0.29e	10 ± 0.26d	10 ± 0.27f	11 ± 0.29d	0.0	11 ± 0.29d	0.0	11 ± 0.29d	11 ± 0.29c	12 ± 0.32d
8 ± 0.21h	8 ± 0.21f	7 ± 0.19i	8 ± 0.21 fg	0.0	8 ± 0.21g	0.0	9 ± 0.24f	7 ± 0.19g	9 ± 0.24g
8 ± 0.21h	0.0	7 ± 0.18i	7 ± 0.18g	0.0	8 ± 0.21g	0.0	9 ± 0.24f	7 ± 0.18g	8 ± 0.21h
10 ± 0.26f	0.0	11 ± 0.29e	12 ± 0.32d	11 ± 0.29d	10 ± 0.26e	10 ± 0.26c	11 ± 0.29d	10 ± 0.26d	11 ± 0.29e
9 ± 0.24g	0.0	9 ± 0.24g	9 ± 0.24ef	7 ± 0.18h	8 ± 0.22g	9 ± 0.24d	10 ± 0.26e	8 ± 0.21f	8 ± 0.21h
8 ± 0.21h	0.0	9 ± 0.24g	70 ± 1.8a	7 ± 0.18h	8 ± 0.22g	8 ± 0.21e	9 ± 0.24f	8 ± 0.21f	8 ± 0.24h
8 ± 0.21h	0.0	0.0	10 ± 0.26de	10 ± 0.26e	7 ± 0.18h	0.0	9 ± 0.23f	0.0	10 ± 0.26f
7 ± 0.18i	0.0	0.0	8 ± 0.21 fg	9 ± 0.24f	7 ± 0.19h	0.0	9 ± 0.22f	0.0	9 ± 0.25g
7 ± 0.18i	0.0	0.0	8 ± 0.21 fg	9 ± 0.24f	7 ± 0.17h	0.0	9 ± 0.21f	0.0	9 ± 0.24g
8 ± 0.21h	0.0	0.0	10 ± 0.26de	8 ± 0.21g	7 ± 0.18h	0.0	0.0	0.0	9 ± 0.24g
8 ± 0.21h	0.0	0.0	8 ± 0.21 fg	8 ± 0.21g	7 ± 0.18h	0.0	0.0	0.0	9 ± 0.24g
8 ± 0.21h	0.0	0.0	9 ± 0.24ef	8 ± 0.21g	7 ± 0.18h	0.0	0.0	0.0	9 ± 0.24g
8 ± 0.21h	0.0	0.0	0.0	9 ± 0.24f	0.0	0.0	0.0	0.0	0.0
8 ± 0.21h	0.0	0.0	0.0	9 ± 0.24f	0.0	0.0	0.0	0.0	0.0
8 ± 0.21h	0.0	0.0	0.0	9 ± 0.24f	0.0	0.0	0.0	0.0	0.0
8 ± 0.21h	0.0	0.0	8 ± 0.21 fg	7 ± 0.19h	7 ± 0.18h	0.0	0.0	0.0	0.0
7 ± 0.18i	0.0	0.0	8 ± 0.21 fg	7 ± 0.19h	7 ± 0.18h	0.0	0.0	0.0	0.0
8 ± 0.21h	0.0	0.0	8 ± 0.21 fg	7 ± 0.19h	7 ± 0.18h	0.0	0.0	0.0	0.0
19 ± 0.51a	20 ± 0.53a	18 ± 0.47a	16 ± 0.42b	19 ± 0.51a	20 ± 0.53a	13 ± 0.34a	17 ± 0.45a	16 ± 0.42a	20 ± 0.53a
12 ± 0.32d	13 ± 0.35b	16 ± 0.42b	14 ± 0.37c	12 ± 0.34c	16 ± 0.42b	0.0	15 ± 0.39b	0.0	12 ± 0.31d
9 ± 0.23g	9 ± 0.23e	8 ± 0.21h	8 ± 0.21 fg	9 ± 0.24f	10 ± 0.26e	0.0	10 ± 0.26e	0.0	10 ± 0.26f
0.0	0.0	0.0	9 ± 0.22ef	11 ± 0.29d	8 ± 0.21g	11 ± 0.29b	0.0	0.0	9 ± 0.26g
0.0	0.0	0.0	7 ± 0.18g	10 ± 0.26e	8 ± 0.22g	9 ± 0.24d	0.0	0.0	9 ± 0.25g
0.0	0.0	0.0	0.0	8 ± 0.21g	-ve	9 ± 0.25d	0.0	0.0	9 ± 0.24g
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10 ± 0.26f	0.0	10 ± 0.26f	0.0	0.0	0.0	0.0	10 ± 0.26e	0.0	10 ± 0.26f
9 ± 0.24g	0.0	8 ± 0.21h	0.0	0.0	0.0	0.0	10 ± 0.26e	0.0	9 ± 0.24g
7 ± 0.18i	0.0	9 ± 0.24g	0.0	0.0	0.0	0.0	10 ± 0.26e	0.0	10 ± 0.26f
9 ± 0.24g	0.0	0.0	11.5 ± 0.31d	9 ± 0.24f	10 ± 0.26e	0.0	0.0	0.0	0.0
9 ± 0.24g	0.0	0.0	10 ± 0.26 fg	9 ± 0.24f	10 ± 0.26e	0.0	0.0	0.0	0.0
9 ± 0.24g	0.0	0.0	8 ± 0.21 fg	9 ± 0.24f	8 ± 0.21g	0.0	0.0	0.0	0.0
9 ± 0.24g	8 ± 0.21f	9 ± 0.24g	8 ± 0.21 fg	-ve	8 ± 0.22g	10 ± 0.26c	0.0	0.0	8 ± 0.21h
-ve	-ve	-ve	8 ± 0.21 fg	-ve	8 ± 0.23g	-ve	10 ± 0.26e	-ve	11 ± 0.29e
12 ± 0.32d	10 ± 0.26d	11 ± 0.29e	12 ± 0.32d	10 ± 0.26e	11 ± 0.29d	-ve	11 ± 0.29d	-ve	12 ± 0.32d

neighbour joining and farthest parsimony in MEGA6 (Tamura et al., 2013).

2.8. Antimicrobial activity of different natural plants against selected MDR Salmonella serotypes

2.8.1. Preparation of plant extracts

Eleven floral plant species were collected from Sharkia and Kafr El-Sheikh governorates along road sides and fields, *Allium cepa, Allium sativum, Moringa oleifera, Albizzia lebbeck, Euphorbia tirucalli, Urtica dioica, Lawsonia inermis, Portulaca oleracea, Ocimum basilicum, Pluchaediscordis and Rahmnus cathertica,* in addition to three commercial powder extracts; Allicin*, Echinacea purpura* and *Actaera cemosa* (Black COHOSH). Twenty grams of each dried powdered plant material was steeped in 100 ml of refined distilled boiled water and certain organic solvents (80% methanol, Methylene Chloride and 94% Ethanol for Black COHOSH only) in a sterile clean cone shaped flagon for 48 h with ceaseless shaking. After filtration through 8 layers of muslin fabric and centrifuged at 5000g for 10 min the supernatant was gathered and focused (in broiler at 45 °C) (Al-Daihan et al., 2013). All extracts were sterilized by filtration through bacterial filter of pore size 0.45 μm (Hydrophilic PTFE- Millipore Millex-LCR, Germany).

2.8.2. Determination of antibacterial activity of the medicinal plant extracts against multi-drug Salmonella serotypes

The antibacterial vitalities of plant essences were established against 14 multi-drug resistant *Salmonella* serotypes, utilizing agar diffusion technique. Sterile filter paper discs (Whatman No.3, 6 mm diameter) were impregnated by plant concentrates and permitted to dry for 1 h then placed on the surface of inoculated agar plates. After incubation, the whole diameters of the restraint zones were estimated including the distance across of the plate (6 mm disk) (Korcan et al., 2013).

2.8.3. Influence of combination between entire plant extracts against selected multi drug resistant Salmonella serotypes

Each methanolic plant extract was tested in combination with each one of the rest, and selected the most effective double combination extracts for preparing of triple combinations to increase the inhibition efficiency of plant extracts against the tested MDR serotypes. The most active methanolic plant extracts; *Allium cepa and Allium sativum* (the highest active double combination extracts) were tested in combination with each other and the rest plant extracts, against 14 selected MDR *Salmonella* serotypes by using Well diffusion method according to Jouda et al. (2016). The inoculums of mixtures were prepared in ratio of (1:1:1 μL) where, every mixture was composed of 500 μL (*Allium cepa*) extract, 500μ (*Allium sativum*) extract and 500 μL of each other plant

Table 4
Effect of triple mixtures of different plant extracts against selected multidrug resistant *Salmonella* isolates using agar-well diffusion method.

Plant extracts Triple Mixtures	Inhibition zone diameter (mm)													
	F24	ChI51	Eg77	ChI87	Eg103	BrL104	BrL106	BrL108	BrL112	Eg113	BrL115	BrL116	Alb. 118	BrL120
A. cepa + *A. sativum* + *P. discordis*	9 ± 0.24d	15 ± 0.41bc	0.0	16 ± 0.42b	15 ± 0.41b	0.0	14 ± 0.37bc	0.0	0.0	0.0	0.0	14 ± 0.37cd	0.0	15 ± 0.39cd
A. cepa + *A. sativum* + *A.lebbeck*	16 ± 0.42b	0.0	0.0	15 ± 0.41c	15 ± 0.39b	0.0	0.0	17 ± 0.45bc	17 ± 0.45b	15 ± 0.39c	0.0	15 ± 0.39bc	0.0	16 ± 0.42c
A. cepa + *A. sativum* + *E.tirucalli*	0.0	0.0	0.0	11 ± 0.29d	10 ± 0.26d	0.0	0.0	10 ± 0.26e	0.0	11 ± 0.29e	0.0	0.0	0.0	8 ± 0.22f
A. cepa + *A. sativum* + *P. oleracea*	0.0	11 ± 0.29f	0.0	10 ± 0.26e	0.0	0.0	0.0	10 ± 0.26e	12 ± 0.32 fg	10 ± 0.26e	11 ± 0.29d	0.0	0.0	10 ± 0.26e
A. cepa + *A. sativum* + *L. inermis*	23 ± 0.61a	26 ± 0.69a	20 ± 0.53a	30 ± 0.79a	29 ± 0.77a	31 ± 0.82a	32 ± 0.84a	31 ± 0.82a	31 ± 0.82a	32 ± 0.84a	22 ± 0.58a	33 ± 0.87a	28 ± 0.74a	29 ± 0.76a
A. cepa + *A. sativum* + *U.dioica*	0.0	14 ± 0.37cd	0.0	10 ± 0.26e	15 ± 0.41b	0.0	15 ± 0.39b	16 ± 0.42c	16 ± 0.42bc	17 ± 0.45b	0.0	15 ± 0.39bc	0.0	15 ± 0.39cd
A. cepa + *A.*														

sativum + *O. basilicum*0.012 ± 0.32ef0.00.013 ± 0.34c0.010 ± 0.26e11 ± 0.29e12 ± 0.32 fg15 ± 0.39c0.015 ± 0.39bc0.00.0*A. cepa* + *A.*
sativum + *R. cathartica*0.012 ± 0.32ef0.00.014 ± 0.37bc0.012 ± 0.32d16 ± 0.42c15 ± 0.39cd15 ± 0.39c12 ± 0.32c13 ± 0.34d12 ± 0.32e14 ± 0.37d*A. cepa* + *A. sativum* + *M. oleifera*
leef0.014 ± 0.37cd0.00.010 ± 0.26d0.013 ± 0.34cd14 ± 0.37d15 ± 0.39cd14 ± 0.37cd12 ± 0.32c15 ± 0.38bc14 ± 0.37d16 ± 0.42c*A. cepa* + *A. sativum* + *M. oleifera*
seed0.013 ± 0.34de0.00.014 ± 0.37bc0.013 ± 0.34cd14 ± 0.37d11 ± 0.29g14 ± 0.37cd11 ± 0.29d16 ± 0.42b15 ± 0.39c16 ± 0.43c*A. cepa* + *A. sativum* + *Echinacea*
extract0.012 ± 0.32ef0.00.010 ± 0.26d0.010 ± 0.26e11 ± 0.29e13 ± 0.34ef13 ± 0.34d11 ± 0.29d15 ± 0.37bc11 ± 0.29f16 ± 0.42c*A. cepa* + *A. sativum* + Black
COHOSH0.013 ± 0.34de0.00.013 ± 0.34c0.013 ± 0.34cd13 ± 0.34d11 ± 0.29g0.00.00.00.014 ± 0.37d*A. cepa* + *A.*
sativum + Allicin13 ± 0.34c16 ± 0.42b0.00.015 ± 0.39b0.010 ± 0.26e18 ± 0.47b14 ± 0.37de13 ± 0.34d13 ± 0.34b0.020 ± 0.53b19 ± 0.51bM: Methanol extraction solvent. *A.* = *Allium.* Black COHOSH= *Actaeracemosa. P. oleracea* = *Portulaca oleracea. L. inermis* = *Lawsoniainermis. U* = *Urtica. O. basilicum* = *Osimumbasilicum. R.* = *Rhamnus. M.* = *Moringa. S.* = *Spirulina. E.* = *Euphorbia. A. lebbeck* = *Albizzia. P. discordis* = *Pluchae.* **Solvent used and ratio of Mixtures (1:1:1 μL).**

Table 5
Detection of different virulence genes determinants in selected resistant virulent *Salmonella* isolates.

Sample	Selected Virulence genes of *Salmonella* spp.					
	*inv*A	*spv*C	stn	*gip*A	*ssa*Q	*sop*B
FA24	+	–	+	–	–	+
CHI51	+	–	+	–	–	+
Eg77 (Egg Yolk)	+	+	+	–	–	+
CHI87	+	–	+	–	–	+
Eg103	+	–	+	–	–	+
BrL104	+	–	+	–	–	+
BrL106	+	–	+	–	–	+
BrL108	+	–	+	–	–	+
BrL112	+	–	+	–	–	+
Eg113 (Shell Egg)	+	-	+	-	-	+
BrL115	+	+	+	–	–	+
BrL116	+	–	+	–	–	+
Alb118	+	+	+	–	–	+
BrL120	+	–	+	–	–	+
% of incidence	100	21.4	100	0.0	0.0	100

(+) = refers to incidence of the target gene. (−) = refers to the target gene is not detected. FA= Feces of Diseased and Healthy. CHI= Chicken Intestine. Eg = Egg Yolk and Shell egg. BrL= Brullus Lake (water sample). Alb = Human Albumin drug. *inv*A = invasion gene. *spv*C = Salmonella plasmid virulence C gene. *Stn* = enterotoxin gene (exotoxin). *gip*A = Peyer's patch-specific virulence factor GipA. *ssa*Q gene = encode for Secretion system apparatus protein SsaQ. *sop*B gene = Inositol phosphate phosphatase SopB.

extract, after which each well filled with 100 μL of final mixture.

3. Results and discussion

In the present study, 120 *Salmonella* serotypes were isolated from 96 different samples. The results showed in Figure (1) demonstrated that the highest numbers of *Salmonella* serotypes were recovered from chicken intestine (40 serotypes) followed by feces of healthy and diseased animals (23 serotypes), and Brullus lake (18 serotypes) with represented percentages of 41.66%, 23.95% and 18.75%, respectively. Meanwhile, the lowest numbers of *Salmonella* serotypes were recovered from clay soil and Human Albumin (drug) with percentages 4.16% and 2.08%, respectively. The highest percentages of *Salmonella* culture isolated from both chicken intestine and brullus lake were 60.4%. *Salmonella* with this percentage represent a potential risk due to the consumption of these chickens, using the lake water or fishing. Hygiene practices is required to reduce the spread of antibiotic-resistant microorganisms and ensure good food. Food and Drug Administration of U.S. A. reported that the infectious dose of *Salmonella* serotypes in certain foods has been assessed to be as low as one cell, so it is considered to be the second most common cause of foodborne illness (U. S. Food and Drug Administration, 2012). Djeffal et al. (2018) showed the prevalence of *Salmonella* serotypes in 27 surveyed slaughterhouses and poultry houses, concrete walls, roofs and corrugated metal sheet roofs with 34.37% of the 32 poultry farms. Moreover, Liu et al. (2018) pointed that irrigation water may be a transmission vehicle for *Salmonella* serotypes contaminating crops. Liu et al. (2018) estimated the persistence of *Salmonella* serotypes in different environments of surface water.

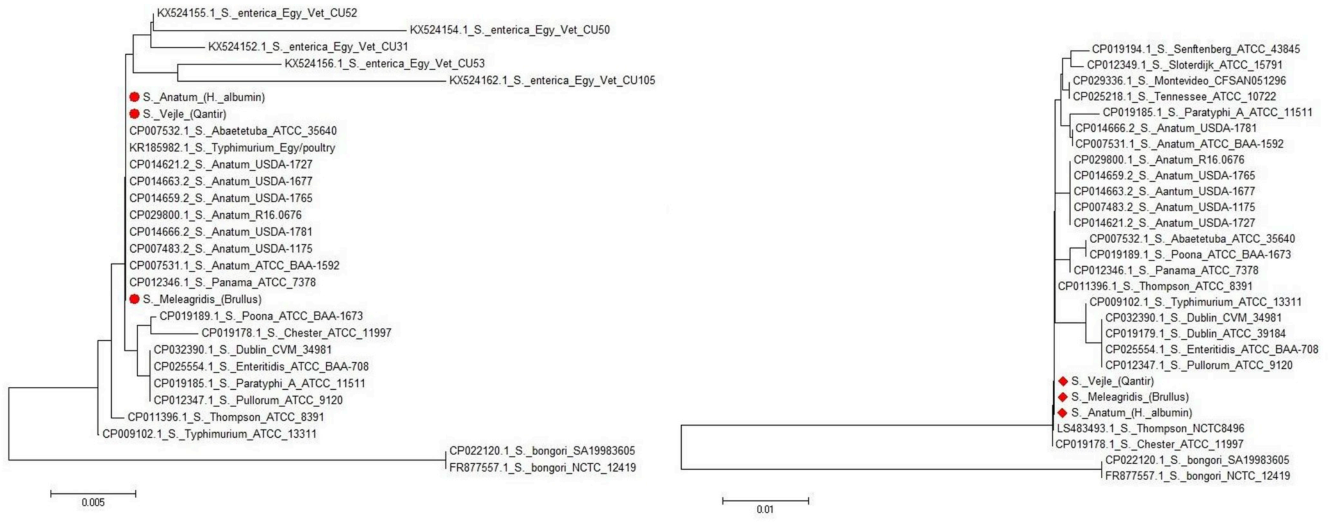
In the current work, all *Salmonella* serotypes were identified by different morphological, physiological and biochemical tests based on the keys of identification protocols of *Salmonella* spp. using different specific selective enriched and pre-enriched growth media (Data not shown). The isolation methods depending on cultures remain the preferable applied techniques at most due to their selectivity and sensitivity for *Salmonella* detection (Alocilja and Radke, 2003). The antibiotic susceptibility test of all isolated *Salmonella* (120 serotypes) against 14 antibiotics, indicated that 14 *Salmonella* serotypes were multi-drug resistant (MDR) to 3 or more different types of entire tested antibiotics. Different antibiotics were chosen as they cover different modes of action against Gram-negative bacteria. The tested serotypes showed 100% susceptibility to ertapenem, nitroFurantoin, ciprofloxacin and norfloxacin followed by sulfametoxazole–trimethoprime with 86.7% susceptibility (Table 2). On the other hand, 100% of total tested bacterial serotypes were resistant to cefaclor followed by Metronidazole, with resistant percentage of 85.0%. Multidrug resistance was characterized as impedance of pathogenic microbes to no less than at least three antimicrobial classes (Centers for Disease Control and Prevention, 2010). The entire selected antibiotics were chosen depending on covering different mechanisms of action of antibiotics (Tarabees et al., 2017). In addition, Gharieb et al. (2015) proposed that human source isolates of *S.* Typhimurium have higher susceptibility to ciprofloxacin (100%) might be because of segregate utilization of this medication since it is moderately costly, not actually reasonable to all individuals and not sold in private drug stores without instruction. Hung et al. (2017) evaluated that 70% out of the 23 disconnects, were vulnerable to ampicillin, 65% were sensitive to ciprofloxacin and 78% were susceptible to trimethoprim-sulfamethoxazole.

As microorganisms turn out to be progressively increasingly impervious to medications of clinical significance, there is an expanded danger of treatment disappointment because of constrained remedial decisions. Non-Typhoidal *Salmonella* plasmid have virulence and antibiotic -resistance determinants simultaneously, which by selected pressure result in more systemic infections and hospitalizations (Rowlands et al., 2014). The developing issue of antimicrobial resistance has brought about a decline in the viability of antimicrobials and a circumstance like the pre-*anti*-microbial period sometimes (WHO, 2011). Therefore, this study is focused on searching of new possibilities resolving this problem. The natural plant extracts can be used as one of the most effective and safe easily method on hygiene and environment for prevention or even decreasing the number of MDR *Salmonella* serotypes. In the current study, 42 methanolic, methylene chloride and aqueous crude extracts, derived from different parts of 11 plant species, were screened for their antibacterial activity against 14 multi-drug resistant *Salmonella* serotypes, in addition to three extracts of Allicin as pure garlic extract, COHOSH and Echinacea as commercial extracts. The obtained results revealed that the maximum inhibitory effect was achieved by *Lawsonia inermis* methanolic extract which recording 16–20 mm inhibition zone diameters and showed the broadest spectrum and effective inhibition between all tested plant extracts against most *Salmonella* serotypes (Table 3). All data revealed that the methanolic extract was better than methylene chloride and aqueous extracts as preferable extraction solvent. Also, double combination of *Allium cepa*

Table 6
Serological Kauffman-white classification (Identification) of the most antibiotic-resistant and virulent *Salmonella* isolates according to WHO manual-antigenic formula of the *Salmonella* serovars.

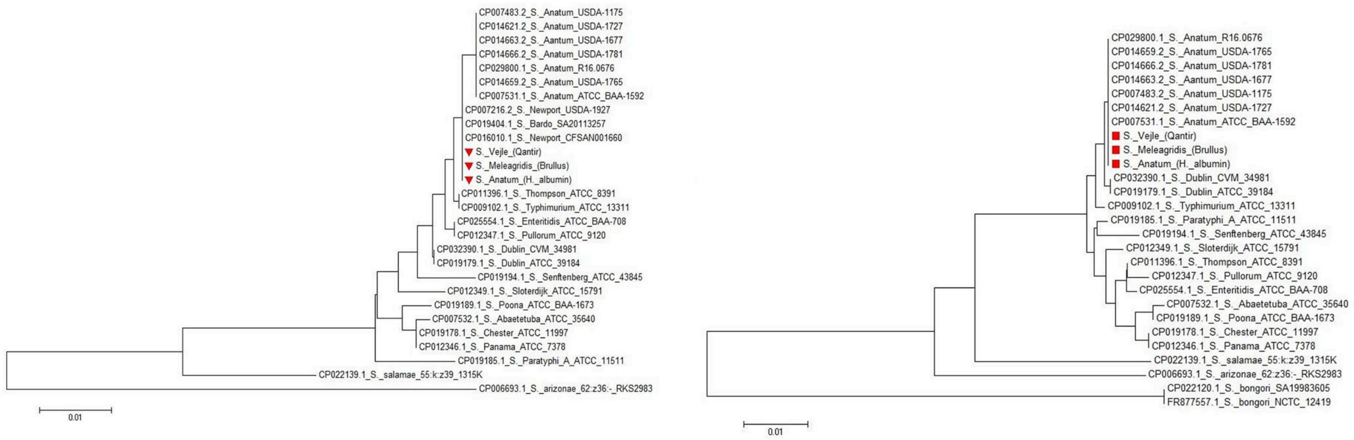
Isolate code	Gram's stain	Genus	species	Subspecies (I)	Serotype	Antigenic structure formula	Family name
Eg77 (Egg yolk)	Gram -ve	*Salmonella*	*enterica*	*enterica*	Vejle	**O3,10; e, h; 1,2**	*Enterobacteriaceae*
BrL115 (Brullus lake) water sample	Gram -ve	*Salmonella*	*enterica*	*enterica*	Meleagridis	**O3, {10}; e, h; l, w**	*Enterobacteriaceae*
Alb.118 (Human Albumin) Drug	Gram -ve	*Salmonella*	*enterica*	*enterica*	Anatum	**O3,10; e, h; 1,6**	*Enterobacteriaceae*

Without certain family [**Bergy's** manual of Determinative Bacteriology, 1977). Eight Edition, Holt Editor]. Isolates code: Eg (egg yolk). 115Br (Brullus Lake). 118Alb (Human albumin for infusion drug).



(a) *inv*A gene

(c) *spv*C gene



(b) *sop*B gene

(d) *stn* gene

Fig. 2. Phylogenetic trees of aligned mega clustalW (weighted) of different genes for *Salmonella* Vejle Eg77 strain MK127933 (Qantir), *Salmonella* Meleagridis BrL115 BrL115 strain MK127934 (Brullus) and *Salmonella* Anatum Alb118 strain MK.

and *Allium sativum* fresh extracts introduced some appreciated inhibitory effects against all entire tested *Salmonella* serotypes as a broad-spectrum inhibition. To increase the inhibition activity of plant extracts against experimental *Salmonella* serotypes, each extract was mixed with the mixture of garlic and onion extract. It is obvious from data that the strong active inhibitory anti-Salmonellae mixture was *Allium cepa* + *Allium sativum* + *Lawsonia inermis* extracts, where this mixture revealed good inhibition against all entire selected *Salmonella* serotypes (Table 4). several components, with antimicrobial properties have been found in plant extracts and essential oils (Norajit and Ryu, 2012). Henna is a naturally occurring plant, which arise wild in desolated regions (Muhammad and Mustapha, 1994). Henna (*Lawsonia inermis*) has a wide scope of phytochemical constituents including naphthoquinone derivatives (lawsone; the main constituent and leaf shading specialist), phenolic compounds, flavonoids, aliphatic parts, coumarins, xanthones, tannic acids, triterpenes, sterols and others, for example, mannitol, glucose, amino acids, gallic corrosive, follow components and minerals (Varghese et al., 2010). The society prescription thought about henna as astringent, calming sedative, hypotensive operators and against a cerebral pain, infection and jaundice (Abdulmoneim, 2007). Henna leaves can be useful in treatment of skin maladies, smallpox, spermatorrhea and venereal sicknesses. Their seeds were fruitful towards diarrhea and liver issue. The bark of henna plant was utilized in the treatment of burns, spleen enlargement, calculus, skin issue and leprosy. Root might be valuable for herpes disease, gonorrhea and some anxious aggravation (Chaudhary et al., 2010). Mohamed et al. (2015) presumed that this plant has cancer prevention agent properties. Habbal et al., 2011 henna samples in Oman possess highest antibacterial activity against *P. aeruginosa* (Habbal et al., 2011). Mostafa et al. (2018) estimated that food can be treated with plant extracts which are natural preservatives to control food poisoning and maintain food away of dangerous chemical preservatives. The free radicals are given by quinones organizing complex irreversibly with nucleophilic amino acids in proteins (Tan and Berridge, 2008), which resulting in inhibition of the protein synthesis and loss of function. So, quinones have antimicrobial effects against the pathogenic microbial cells. Quinones make substrates inaccessible to the microorganism (Córdoba-Pedregosa et al., 2006).

Moreover, garlic (*Allium sativum*) is considered as anti-infective agent besides its conventional regimen (Lawson, 1998). Ingesting approximately 50 mg of garlic daily, protect from gastrointestinal troubles (Wolde et al., 2018). Normally developing plants have assumed a vital job in the revelation of new remedial specialists. The remedial uses incorporate helpful impacts on the cardiovascular framework, anti-microbials, anticancer, anti-inflammatory, and hypoglycemic and hormone-like impacts (Shaloo et al., 2015). The essential oils of onion and garlic and their constituent sulfides with at least three sulfur particles were strong powerful inhibition against yeast development (Kim et al., 2004). Garlic is one of the herbs that was utilized by conventional experts for arrangement of herbals drug. Development of drug resistance is evident and worldwide stand up. Allicin is one of the dynamic central of crisply squashed garlic homogenates, which have assortment of antimicrobial exercises. The Antibacterial movement of the unrefined concentrates of garlic was assessed against *S. aureus* and *E. coli* by an agar diffusion technique. *E. coli* were more vulnerable than *S. aureus* to the concentrates. Garlic could be utilized as successful antibacterial specialist against human pathogenic microscopic organisms (Wolde et al., 2018). The onion extricates have bactericidal impacts on *S. mutans*, *S. sobrinus*, *P. gingivalis* and *P. intermedia* societies (Kim, 1997). Expanding groupings of onion remove altogether influenced *E. coli* and yeast-mould checks (Irkin and Arslan, 2010).

The *inv*A, *stn* and *sopB* genes can be used as specific targets for detection of *Salmonella* as they are conserved among the *Salmonella* irrespective of serotype (Ogunremi et al., 2017). The incidence or absence of certain virulence genes illustrating some variation between isolates, indicating that remarkable contribution to the plasticity of the *S. enterica* genome (Figueiredo et al., 2015). Therefore, this study investigated the presence of these genes in addition to *gip*A, *ssa*Q and *spv*C virulence genes in tested *Salmonella* serotypes. The resulting amplicons of PCR using designed specific primers of 6 virulent genes are illustrated in Figures (S1-6). Moreover, the results in Table 5 indicated that the *inv*A, *stn* and *sop*B genes were detected in 100% of the *Salmonella* serovars, meanwhile the *gip*A and *ssa*Q genes haven't been detected in all of the isolated *Salmonella* serovars. Also, *spv*C gene was demonstrated only on three isolates by 21.4% between entire 14 tested *Salmonella* serotypes. The results showed that all isolates suspected to be *Salmonella* spp. contain *inv*A*, stn* and *sop*B genes which meaning that all tested isolates were belonged to *Salmonella* spp. Only three *Salmonella* isolates; Eg77, BrL115 and Alb118 possessed the *inv*A*, stn, sop*B and *spv*C genes (Table 5). These results confirmed positive correlation between antibiotic resistance and virulence genes in *Salmonella*. Chaudhary et al. (2015) revealed that *inv*A and *stn* virulence genes exist in almost *Salmonella* serotypes which perceived as global standard for identification of *Salmonella* family and is in regards to intestinal intrusion. Thung et al. (2018) explored the presence of *stn* gene among *Salmonella* serotypes (69.57%) with lower frequencies. Moreover, *ssa*Q and *gip*A genes were not detected in all serotypes sensitive to flouroquinolone. This result meaning that the resistance patterns of flouroquinolone were associated with these genes. Osman et al. (2014) revealed that *inv*A and *sop*B genes were detected in 100% of the *Salmonella* serovars; while, the *gip*A gene was absent at all. Moreover, our results clarified that *ssa*Q gene was also not detected in our serotypes, these results may help in realizing the virulence genes and antibiotic resistance/susceptibility patterns in Egypt. Occurrence of *inv*A, *sop*B and *stn* gene in all serotypes demonstrates that they are the particular focuses for *Salmonella* recognizable proof and are fit for creating gastroenteric sickness to people, whereas the three selected *Salmonella* Vejle, Meleagridis and Anatum serovars can be able to produce systemic infection especially in some cases results in death. Interestingly, our study also recommended that *inv*A and *stn* virulence genes are highly saved in *S. enterica,* isolated from commercial food stuffs and could be utilized autonomously as a quality marker for the fast recognition of the destructive strains of *S. enterica*. The prevalence of *spv*C gene is restricted into the serotypes of a few definite sources (Borges et al., 2013). So, our results revealed the *spv*C gene incidence disparity which may innervate its rotation among the plasmid and chromosomal factors, depending on the genetic profile of each isolate (Borges et al., 2013). Many serovars carrying *spv* locus were associated with human disease such as, Typhimurium, Enteritidis, Choleraesuis, Dublin, and Arizona (Krause et al., 1992). These results revealed that different *Salmonella* serovars may or not share the same virulence genes despite of isolation from different sources depending on the host meaning that wide distribution and genetic diversity of *Salmonella enetrica* serotypes.

The isolates containing the four virulence genes were selected and further identified by studying their serotypes to ***Salmonella* Vejle** Eg77 (egg yolk), *Salmonella* Meleagridis BrL115 (Brullus lake) and *Salmonella* Anatum Alb118 (Human albumin drug) (Table 6). The antigenic varieties among the 46 *Salmonella* O serogroups are expected chiefly to hereditary variety groups in their respective *rfb* gene clusters (Xiang et al., 1993). Blast analysis showed that the amplified genes of *Salmonella* were closely related to the other virulence genes of *Salmonella* serovars deposited in the GenBank. All analyzed genes were submitted in GenBank under the accession numbers reported in Table (1). The phylogenetic trees of tested genes in three selected *Salmonella* serovars were shown in Fig. 2.

4. In conclusion

It concluded from this study that a combination of plant extracts of *Allium cepa, Allium sativum* and *Lawsonia inermis* have potent antibacterial activity against *Salmonella* growth compared to ertapenem, nitrofurantoin antibiotics. This mixture extract can be used in controlling of *Salmonella* instead of chemical antibiotics. Also, it concluded from this study that all selected pathogenic strains have the four virulence genes which confirm their identification.

Conflicts of interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101350.

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