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Susceptibility of *Salmonella* Serovars Recovered from Hospitalized Saudis to Commercial Drugs and to a Chemically and Safety-Characterized Essential Oil Blend

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Abstract

Background

This study aimed at the identification of the *Salmonella* serovars involved in human salmonellosis outbreaks in Saudi Arabia, determination of their susceptibility to drugs, and determination of Minimum Inhibitory Concentration (MIC) of an essential oil-blend of eucalyptus and peppermint.

Material and Methods

The identification of the O and H antigens of the *Salmonella* serovars was according to WHO protocol, while their susceptibility to active ingredients of 23 drugs was accomplished by single disk diffusion assay. The MIC was determined by a standard protocol, while the safety of the blend was assessed by Draize Eye and Ames tests.

Results

The distribution of the 16 separately admitted Saudi patients, due to their infection with different *Salmonella* serovars were: 4 (*S.enteritidis*), 4 (*S. typhimurium*), 3 (*S. kentucky*), 3 (*S. anatum*), and 2 (*S. typhi*). The three in vitro effective drugs against all 14 non-typhoid isolates were Cefepime, Chloramphenicol, and Norfloxacin, while 10 drugs were effective against S.typhi isolates. A positive correlation (R=0.46, P less than 0.05) between the frequency of drugs that the *Salmonella* isolates were resistant to and the MIC of the essential oil-blend was observed ($P<0.05$). The essential oil blend was found to be safe for use.

Conclusion

Only three drugs out of 23 were effective *in vitro* against the Non-Typhoids, while 10 drugs were fortunately effective against the Typhoids. The safety and MIC data related to the novel blend of the essential oils encourage to pursue its efficacy *in vivo* to help for future use against *Salmonella* infections.

Keywords: Commercial drugs; Essential oil; MIC; Salmonella; Saudi Arabia; Serovars; Susceptibility

Introduction

Most developed countries have a system of annual reporting of the frequency of recovered *Salmonella* serovars from human and other animal hosts [1]; however, most developing countries rely on sporadic reporting, based on targeted surveillances of *Salmonella* outbreaks [2]. The pathogenicity of the differentserovars in human and different animal hosts varies significantly, allowing to branch Salmonellosis into different diseases, including Typhoid in human, caused by *S. Typhi* [3], non-typhoids in human caused by serovars other than *S. Typhi* [4], Fowl Typhoid and Bacillary White Diarrhea disease in poultry caused by *S. Gallinarum* and *S.Pullorum*, respectively [5], and paratyphoid infections in human and many species of animals, caused by 100(s) of other serovars [4].

Only ten of the developed countries present to their public, on their specialized websites, annual reports related to the susceptibility of recovered *Salmonella* serovars to important drugs used in medical and veterinary practices [6]. Unfortunately, most developing countries, including the ones with huge population size, have a paucity of these reports [7].The physicians and veterinarians of the developed countries rely routinely on these web-available reports for a better understanding of the shift in efficiency of the available drugs against *Salmonella* organisms; however, the paucity of these reports in most developing countries deprive the medical and veterinary communities from vital directions to their chemotherapeutic practices [2].

The nowadays resistance of *Salmonella* serovars to drugs is widespread across the globe, including resistance to antimicrobials that were the 'drugs-of-choice', since a decade [8]. Accordingly, new members of drugs, under the original generations, are synthesized and marketed, aiming to provide wide leverage of efficiency to prescribed treatments by physicians and veterinarians against Salmonellosis [9].

The most common drugs experimented on against gram-negative bacteria were 13 cell wall inhibitors namely, Amoxicillin/Clavulanic acid, Ampicillin, Aztreonam, Cefamandole, Cefepime, Cefixime, Cefotaxime, Cefoxitin, Ceftazidine, Cefuroxime Sodium, Cephalothin, Imipenem, and Piperacillin/Tazobactam [10]. In addition, the most searched six drugs inhibiting the protein synthesis in bacteria were Amikacin, Chloramphenicol, Gentamicin, Kanamycin, Tetracycline, and Tobramycin [11], while those inhibiting the nucleic acid synthesis were three quinolones namely, Ciprofloxacin, Nitrofurantoin, and Norfloxacin [12], and the one folate pathway-inhibitor was the bivalent synergist of Sulfamethoxazole/Trimethoprim [13].

Due to the frequent occurrence of *Salmonella* serovars with resistance to multiple synthetic drugs, World Organizations (WHO, OIE, and EFSA) and individual researchers [14] are continuously investigating the presence of antimicrobial activities in herbs [15], biofilms on sea algae, and various metabolic products [16]. This trend in research emerged as a result of documented surveillances, presenting data on multiple drug resistance in different *Salmonella* serovars [17]. A blend of chemically-characterized eucalyptus and peppermint essential oils (1/1, v/v ratio) has been subjected to various investigations, including the evaluation of its activity against viral [18], protozoal [19], and bacterial [20] etiologies of economic diseases, with an absence of data related to safety.

To our knowledge, this is the first work that aimed at the identification of the Salmonella serovars involved in outbreaks that led to hospitalization of Saudi Arabian patients due to typhoid and non-typhoid conditions. In addition, this work investigated the susceptibilities of the identified human serovars to 23 active ingredients of the most common commercial drugs, including control isolates with known multiple drug-resistance. Moreover, the MIC of the essential oil blend against isolates of all recovered serovars was determined, attempting to correlate their values to the frequency of resistance to the 23 commercial ingredients. The safety of the essential oil blend on mucosal membranes and its carcinogenic potential were assessed by Rabbit-Draize Eye and Ames tests, respectively.

Methods and Materials

Serovar Identification

The human *Salmonella* isolates were provided to the Biochemistry Department at King Abdulaziz University in Jeddah, by hospitals in the Kingdom of Saudi Arabia, associated with their accessions, confirming the admittance of Saudi patients for fecal sampling at different times of salmonellosis incidents. The serovar identification of the recovered *Salmonella* from the fecal samples was performed according to the WHO protocol [21], using the sera for serogrouping, somatic and flagellar antigen-identification, and for phase conversions, purchased from Statens Serum Institution Ab-Salmonella, located at 5 Artillerivej 2300 Cph.S, Denmark.

Susceptibility of *Salmonella* Serovars to 23 Drugs

Two control *Salmonella gallinarum* isolates, with wide spectrum of drug-resistance, were included in the susceptibility testing of the human *Salmonella* serovars recovered from Saudi patients. The protocol used in testing the isolates' susceptibility was a single-disk-diffusion test [22].The 23 different antimicrobial disks were purchased from Oxoid ltd, Basingtoke, Hampshire, England. The disks and their potencies are listed below, according to their mode of antibacterial inhibition:

Cell Wall Inhibitors

Amoxicillin/Clavulanic acid (30 mcg), Ampicillin (10 mcg), Aztreonam (30 mcg), Cefamandole (30 mcg), Cefixime (5 mcg), Cefotaxime (30 mcg), Cefoxitin (30 mcg), Ceftazidime (30 mcg), Ceforoxime Sodium (30 mcg), Cephalothin (30 mcg), Imipenem (10 mcg), Cefepime (30 mcg), and Piperacillin/Tazobactam (85 mcg).

Protein Synthesis Inhibitors: Amikacin (30 mcg), Chloramphenicol (30 mcg), Gentamicin (10 mcg), Kanamycin (30 mcg), Tetracycline (30 mcg), and Tobramycin (10 mcg).

Nucleic Acid Synthesis Inhibitors: Ciprofloxacin (5 mcg), Nitrofurantoin (300 mcg), and Norfloxacin (10 mcg).

Folate Pathway-Inhibitors: Sulfamethoxazole/Trimethoprim (25 mcg).

MIC of the Essential Oil Blend

The chemically-characterized essential oil blend was provided by EWABO Chemikalien GmbH & Co, Wietmarschen, Germany. The essential oil was a blend of eucalyptus and peppermint (1/1, v/v). The percentages of the main active ingredients, provided by EWABO Co., were: 1,8-cineol (8%), Menthol (4%), Menthone (2.0-4.6%), Pinene (0.1-1.0%), Phellandrine (0.1-1.0%), Limonene (0.1-1.0%), gamma-Terpinene (0.1-2.0%), Methyl acetate (0.3-1.0%), and Methofuran (0.3-1.0%). This blend is chemically-engineered to form an oil-in-water emulsion, enhancing its homogenous dispersion in aqueous diluent. The MIC protocol followed a previously documented procedure [23], with dilutions of the blend ranging between 0.02 to 8.0%. Briefly, each dilution of the blend was incorporated in triplicates of Tryptose Phosphate broth medium, followed by inoculation of the *Salmonella* serovar culture and incubation overnight at 37°C. The MIC values were recorded according to the minimum dilution of the blend incorporated in the medium that enables the growth-inhibition of the *Salmonella* culture.

Correlation of Blend-MIC to Drug-Resistance

The multiple regression analysis (SPSS v.22, SPSS Inc., Chicago, IL 2015) was performed to determine the level of significance in the correlation between the MIC values of the essential oil blend against the recovered *Salmonella* isolates to the number of drugs that these isolates were resistant to. The level of significance of the correlation is determined at $P<0.05$.

Safety Assessment of the Blend

The safety assessment of the essential oil blend was determined by Rabbit-Draize Eye [24] and Ames tests [25].

Rabbit-Draize Eye Test

The safety of the essential oil blend, in its inability to induce conjunctival inflammatory reaction, was assessed by the Rabbit-Draize Eye test [24]. Briefly, nine male rabbits of one year old were divided evenly into three treatments. Rabbits of Treatment 1 were the controls, deprived of any application of the blend on their eyes. Rabbits of Treatment 2 received a 2% dilution of the blend, in a volume of 50 µl/each of their left eye, and repeating the application for three consecutive days. Rabbits of Treatment 3 received a 6% dilution of the blend in a similar manner to that used in Treatment 2. The right and left eyes of the 9 rabbits were examined daily and for a period of 7 days, effective the first day of application. The eye examination included the observation of inflammation, manifested in redness, swelling, discharge, ulceration, hemorrhaging, cloudiness, and blindness.

Ames Test

The procedure of the Ames test, applied on the essential oil blend, was adopted from previously documented protocols [26]. Briefly, the test organism was *Salmonella enterica subsp. Enterica serovar typhimurium* (ATCC ® 29629-Strain Designations: TA 1535), an auxotrophic mutant, provided by ATCC, Manassas, USA. The provided mutant requires histidine for growth. The test of mutagenesis involves the addition of the blend at

different dilutions (0.02, 2.0, 3.0, 4.0, 5.0, and 6.0 %) to a histidine-free medium [25], and observing the ability of the blend to revert back the mutated *S.typhimurium* to a prototrophic state, enabling it to synthesize histidine and to grow. The negative control medium was not supplemented with the oil blend, while the two control-positive media were supplemented with either 20 or 200n moles of the mutagen Ethidium Bromide. The *S.Typhimurium* mutant was plated in triplicates on each of the previously described media, incubated at 37°C for 24 hrs, and the number of colonies was recorded to calculate the % increase in colony count in the medium supplemented with the blend in relation to that growing on non-supplemented medium, using the Ethidium Bromide as a positive control mutagen.

Research Ethics

The conducted study was approved by the Unit of Biochemical Ethics Research Committee, King Abdulaziz University (KAU), reference number 36-14.

Results

The frequency of effective antimicrobials, according to their mode of action, and the MIC values of the essential oil blend against different isolates of each *Salmonella* serovar recovered from Saudi patients are presented in Table 1. The range of frequencies of drugs against the *S. Enteritidis* isolates are bracketed as follows: cell wall inhibitor-drugs (1/13-8/13), protein synthesis inhibitors (0/6-2/6), nucleic acid synthesis inhibitors (0/3-1/3) and folate pathway inhibitor (0/1-1/1). This apparent variability in susceptibility of *S.Enteritidis* to drugs was associated with a wide range of the blend MIC values against isolates of this serovar, equivalent to dilutions from 0.64 to 8.0%.

Table 1 also showed the ranges of frequencies of drugs that are effective against the *S.Typhimurium* isolates. The ranges of frequencies were: cell wall inhibitors (4/13-7/13), protein synthesis inhibitors (0/6-2/6), nucleic acid synthesis inhibitors (0/3-2/3), and absence of sensitivity to the folate inhibitor. The blend MIC values against the *S.Typhimurium* isolates ranged between 2.0-8.0%.

The effective ranges of frequencies of drugs against the *S.Kentucky* isolates were: cell wall inhibitor-drugs (0/13-3/13), protein synthesis inhibitors (0/6-1/6), nucleic acid synthesis inhibitors (0/3-1/3), and absence of sensitivity to the folate inhibitor. The MIC of the essential oil blend against the *S.Kentucky* was always > 8.0%.

The ranges of effective frequencies of drugs against the *S.Anatum* were: cell wall inhibitors (0/13-6/13), protein synthesis inhibitors (0/6-2/6), nucleic acid synthesis inhibitors (0/3-2/3), and folate acid inhibitor (0/1-1/1). The MIC of the essential oil blend against the *S.Anatum* was always > 8.0%.

The ranges of effective frequencies of drugs against the *S.Typhi* isolateswere: cell wall inhibitor-drugs (5/13-7/13), protein synthesis inhibitors (1/6), nucleic acid synthesis inhibitors (0/3-2/3), and folate acid inhibitor (0/1-1/1). The MIC of the essential oil blend against the *S. Typhi* isolates was of wider range namely, 0.32 to more than 8.0%.

Table 1. The ranges of effective antimicrobial-frequencies and MIC values of essential blend against isolates1 of *Salmonella* serovars recovered from Saudi patients

Antimicrobials[2]	Range of effective antimicrobial frequencies or MIC[3] values against isolates of				
	S. Enteritidis	*S. Typhimurium*	*S. kentucky*	*S. Anatum*	*S. Typhi*
Cell Wall inhibitors	1/13 - 7/13	4/13 - 7/13	0/13 - 3/13	0/13 - 6/13	5/13 - 7/13
Protein synthesis inhibitors	0/6 - 2/6	0/6 - 2/6	0/6 - 1/6	0/6 - 2/6	6-Jan
Nucleic acid synthase inhibitors	0/3 - 1/3	0/3 - 2/3	0/3 - 1/3	0/3 - 2/3	0/3 - 2/3
Folate pathway inhibitor	0/1 - 1/1	0/1	0/1	0/1 - 1/1	0/1 - 1/1
MIC ranges of Essential oil blend	0.6 - >8.0	2.0 - 8.0	>8.0	>8.0	0.32 - >8.0

[1]The bracketed numbers of isolates, tested for their antimicrobial susceptibility, and affecting individual patients with specific serovars are: S. Enteritidis (4), S. Typhimurium (4), S. Kentucky (3), S. Anatum (3), and S. Typhi (2)

[2]The bracketed numbers of tested antimicrobials, under each mechanism of activity are: cell wall inhibitors (13), protein synthesis inhibitors (6), nucleic acid inhibitors (3), and folate pathway inhibitor (1)

[3]The MIC value is the minimum dilution of the essential oil blend that inhibits the growth of a Salmonella isolate

It is worth noting that the two control-poultry Salmonella isolates, recovered from poultry of Brazil and Nigeria, with long history of antimicrobial applications in feed and drinking water, were confirmed with high multiple drug-resistance (Table 2).Only one out of 23 drugs was effective *in vitro* against the Brazilian *S.Gallinarum* isolate involved in economic fowl typhoid outbreaks, while no drug out of the 23 was effective against the Nigerian isolate of *S. Gallinarum*. Fortunately, the MIC of the blend against both control isolates was low (0.32%).

Table 3 shows the most effective drugs against different serovars of *Salmonella* involved in human outbreaks in Saudi Arabia. Among the 13 cell wall synthesis inhibitors, only the Cefepime was effective against all human non-typhoid and typhoid serovars. The chloramphenicol was persistent as the drug-of-choice among protein synthesis inhibitors against the human non-typhoid serovars, but not against the typhoid isolates, in which Amikacin and Tobramycin were the effective protein synthesis inhibitors of all typhoid cultures. The Norfloxacin was the most effective nucleic acid inhibitor of non-typhoid and typhoid isolates, while the frequency of the folate-inhibitor effectiveness was scarce against both the non-typhoid and typhoid isolates.

Table 2. The frequency of effective antimicrobials and MIC values of the essential oil blend against the control S. Gallinarum isolates recovered from Brazilian and Nigerian poultry farms with long history of antimicrobial administration

Antimicrobials [1]	Frequency of effective antimicrobials or MIC[2] values against *S. Gallinarum* recovered from poultry of	
	Brazil	Nigeria
Cell Wall inhibitors[3]	1/13	0/13
Protein synthesis inhibitors	0/6	0/6
Nucleic acid synthesis inhibitors	0/3	0/3
Folate pathway inhibitor	0/1	0/1
MIC of Essential oil blend	0.32	0.32

[1]The bracketed numbers of tested antimicrobials, under each mechanism of activity, are: cell wall inhibitors (13), protein synthesis inhibitors (6), nucleic acid inhibitors (3), and folate pathway inhibitor (1)

[2]The MIC value is the minimum dilution of the essential oil blend that inhibits the growth of a Salmonella isolate

[3]The only cell wall inhibitor that is effective against the Brazilian S. gallinarum isolate was the Imipenem

Table 3. The most effective drugs against different serovars of Salmonella involved in human outbreaks of Saudi Arabia

Salmonella Serovars	Most effective drugs with inhibitory mechanism to synthesis of			
	Cell wall	Protein	Nucleic acid	Folate
Human-Non Typhoids				
S. Enteritidis	Cefepime Imipenem	Chloramphenicol	Norfloxacin	SXT1
S. Typhimium	Cefepime	Gentamicin Chloramphenicol	Ciprofloxacin Norfloxacin	None
S. Kentucky	Cefepime	Chloramphenicol	Norfloxacin	None
S. Anatum	Cefepime	Chloramphenicol	Norfloxacin Ciprofloxacin	SXT
Human-Typhoid				
S. Typhi	Cefepime Cefoxitine Ceftazi-dineAztreonam Imipenem	Amikacin Tobramycin	Norfloxacin Ciprofloxacin	SXT

[1]Sulfamethoxazole/Trimethoprim

The correlation, using multiple regression analysis, between the MIC values of the essential oil blend and the frequency of drugs that are effective against the human *Salmonella* isolates is shown in the R^2 value of +0.46, with a significant correlation at $p<0.05$.

The repeated application of the 2 and 6% dilutions of the essential oil blend on the rabbit's eyes did not result in any gross inflammation lesion.

In addition, the Ames test proved the absence of mutagenesis on the test organism of *S.Typhimurium* at concentrations between 0.02-4.0% (Table 4). However, induced mutations by the blend started weakly at 5% dilution, and raised sharply at 6%. The positive control, Ethidium Bromide supplemented medium; showed a clear mutagenesis at 200 nmole but not at a lower concentration of 20 nmole.

Table 4. Mutagenicity[1] in histidine-deficient S. Typhimurium[2] by supplementation of its growth medium with different dilutions of the essential oil blend

Supplementation of growth medium[3]	Mean[4] % increase in S. Typhimurium colony count compared to its count on minimal histidine-supplemented medium3
EO5- 0.02%	-16.7
EO - 2.0%	-7.7
EO - 3.0%	0
EO - 4.0%	-16.7
EO - 5.0%	16.7
EO - 6.0%	90.9
EB6 - 20 nmole	0
EB - 200 nmole	12.5

[1]Mutagenicity is detected by the ability of the S. Typhimurium mutant to revert back to an organism that can synthesize its own histidine (Ames test)

[2]*Salmonella enterica subsp. Enterica serovar Typhimurium* (ATCC ® 29629-Strain Designations: TA 1535), an auxotrophic mutant, provided by ATCC,Manassas, USA

[3] The growth medium was prepared in reference to Current Protocols in Toxicology (1999)

[4] Mean colony count growing on triplicate plates

[5] EO is the essential oil blend

[6] EB is a control positive substance that induces mutagenesis namely, Ethidium Bromide

Discussion

The wide variation in the frequency of effective antimicrobials against the *S.Enteritidis* isolates recovered from different individual cases (Table 1) is most likely due to the different history of exposure to the drugs by the humans or animals that hosted this serovar [27]. The variation in the frequency of effective antimicrobials against *S.Typhimurium* isolates was narrower, which could be due to the lower rate of infection and exposure to drugs of this serovar in poultry and other animal hosts compared to that of *S.Enteritidis* [28]. Actually, the documented prevalence of *S.Enteritidis* in relation to *S.Typhimurium* in sampled poultry of Saudi Arabia was 10 to 1 [29]. In animal hosts, the frequency of infection by *S. Enteritidis* is higher than by *S. Typhimuriam* [30] an indication of the higher adaptability of *S. Enteritidis* to a wide range of hosts, rendering it more exposed to antimicrobial agents and hence more resistant to drugs [28].Unfortunately, the frequency of effective antimicrobials against the other two non-typhoids (*S.Kentucky* and *S.Anatum*) diminished significantly, resulting in much narrower variation of such frequencies among their isolates. These two non-typhoid serovars are reported previously to infect human in the United States, 1968-2011 [30], with an alarming resistance of *S. Kentucky* to ciprofloxacin [31]. Future investigationin Saudi Arabia should involve an epidemiology study of these two highly drug-resistant serovarsin both animals and human [32] and investigation of their pathogenesis in serious human outbreaks [33].

The higher frequency of effective drugs against the *S.Typhi* recovered from Saudi patients compared to that of the non-typhoids, is most likely due to the inadaptability of this serovar to animal hosts, thus avoiding the pressure of antimicrobial use in different animal species that selects for drug-resistant organisms [3]. A clear example of the impact of overuse of antimicrobials in poultry husbandry on emergence of drug-resistant *Salmonella* is seen in the Brazilian and Nigerian isolates of *S.Gallinarum* (Table 2). The fact that this serovar causes Fowl Typhoid, resulting in flock mortality between 90-100 %, obliges the farmers to overuse different antimicrobials, trying to save the affected flocks, leading to emergence of resistant strains to a wide range of antimicrobials [5].The drugs-of-choice, uncovered in this research, is located under their mode of action in Table 3. The Cefepime, a cell wall inhibitor of the 4th generation cephalosporins, is effective against most serovars targeted in this study. Actually, the high Cefepime efficacy against *Salmonella* is reported from many parts of the world, in which physicians refer to it in treating both the non-typhoid and typhoid human cases [34].

Among the studied protein synthesis inhibitors (Table 3), the chloramphenicol is effective against all isolates of non-typhoid serovars, a fact that is in agreement with the reported high efficacy of this drug against Salmonellosis by WHO. In addition, the Amikacin and Tobramycin-protein synthesis inhibitors are found effective against the isolates of the *S.Typhi*, a data that is in agreement with previous reports related to these two drugs [34].

The studied nucleic acid inhibitors showed that Norfloxacin is the drug-of-choice for these investigated human non-typhoid and typhoid salmonellae. This quinolone is actually rated at the top of the list of antimicrobials used against human Salmonellosis [34].

The presence since more than four decades of Sulfamethoxazole/Trimethoprim combination in the global markets of veterinary and human medicine led to its lower effectiveness against *Salmonella* organisms compared to its high reported efficiency in the eighties [35].The recommendation by the WHO to limit the use of some efficient drugs to humans [34] is nowadays respected by many veterinary communities, in attempts to avoid the emergence of highly drug-resistant *Salmonella* that threatens the public health.

The obtained MIC and safety data (Table 1 and Table 4) related to antimicrobial activity of the essential oil blend against non-typhoid and typhoid salmonellosis is within the second goal of WHO Traditional Medicine Strategy 2014-2023, quoting 'strengthening safety, quality, and effectiveness [36].The wide range of dilutions of the essential oil blend, needed to inhibit different isolates, within most of the same serovars (Table 1), is indicative that the susceptibility of *Salmonella* serovars to essential oils varies in a similar trend as that of their susceptibility to modern synthetic drugs. Actually, the multiple regression analysis did prove the positive correlation between the frequencies of the drugs that the *Salmonella* isolates are resistant to and the MIC values of the essential oil blend ($p<0.05$).

Future investigations should relate the *in vitro* efficacy of the essential oil blend to their *in vivo* efficacy in mice or rat models [37].The studied essential oil blend safety was manifested in the absence of conjunctival inflammation in the rabbit's eyes following multiple application of the essential oil at 2 and 6% dilution. Previous researches confirmed the presence of anti-inflammatory substances in eucalyptus[38] and peppermint oils [39].The protocol of the Ames test used in this study allowed to confine its safety, based on its inability to induce mutagenesis, at the range of dilutions between 0.02 to 4.0 % (Table 4). Actually, the extracts of these two plants are nowadays incorporated in lozenges that are bought over the counter, without prescription by physicians, and are documented as FDA-approved additives in foods [40].

The targeted research related to Salmonellosis in Saudi Arabian patients revealed the presence of both, the typhoid and non-typhoid isolates. The non-typhoid cases were dominated by *S.Enteritidis* and *S.Typhimurium* serovars; all non-typhoid isolates were sensitive to Cefepime, Chloramphenicol, and Norfloxacin. Patients with typhoid organisms had more leverage of treatments, since their isolates were sensitive to 10 out of 23 tested antimicrobials.

The *in vitro* efficiency of the essential oil blend was dependent on its dilution. Moreover, the absence of induction of conjunctival inflammation and mutagenesis by the essential oil blend weredetermined at 4.0 and 6.0%, respectively.

Conclusion

Only three drugs out of 23 were effective *in vitro* against the Non-Typhoids (Cefepime, Choloramphenicol, and Norfloxacin), while 10 drugs were luckily effective against the Typhoids. The safety and MIC data related to the novel blend of the essential oils encourage to pursue its efficacy *in vivo* in an attempt to help in future therapy of *Salmonella*-infected animals and humans.

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