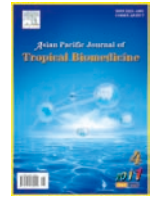




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi:10.1016/S2221-1691(11)60040-3 © 2011 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Isolation and identification of *Salmonella* from curry samples and its sensitivity to commercial antibiotics and aqueous extracts of *Camelia sinensis* (L.) and *Trachyspermum ammi* (L.)

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ARTICLE INFO

Article history:

Received 25 February 2011

Received in revised form 18 March 2011

Accepted 3 April 2011

Available online 30 April 2011

Keywords:

Food-borne

Salmonella

Drug resistance

Plant extracts

Curry samples

*Camelia sinensis**Trachyspermum ammi*

Commercial antibiotics

ABSTRACT

Objective: To isolate *Salmonella* from curry samples and to evaluate the drug sensitivity of the food-borne *Salmonella* and its susceptibility to specific plant extracts. **Methods:** *Salmonella* was isolated from the curry samples by standard microbiological methods and was confirmed by biochemical tests. The antibiotic susceptibility test was conducted by disc diffusion method using commercially available antibiotics such as ampicillin, tetracycline, chloramphenicol, kanamycin, and penicillin. In addition, the susceptibility of the food-borne *Salmonella* was also evaluated against the aqueous extracts of *Camelia sinensis* (L.) Theaceae (tea leaves) and the *Trachyspermum ammi* (L.) Apiaceae (ajwain or omum seeds). **Results:** Out of fifty curry samples, only seven samples were identified to have *Salmonella* contamination. The *Salmonella* isolates showed a significant drug resistance pattern except for kanamycin. The plant extracts showed a considerable antibacterial activity against the isolates, indicating the presence of antimicrobial principle which can be exploited after complete pharmacological investigations. **Conclusions:** The present study demonstrates the occurrence of *Salmonella* in the curry samples, and shows significant drug resistance against most of the commercially available antibiotics, except kanamycin. Antimicrobial effect of the plant extracts against the food-borne *Salmonella* suggests that dietary including medicinal herbs would be one strategy to manage food borne pathogens.

1. Introduction

The three leading food-borne pathogens such as *Salmonella*, *Campylobacter* and *Escherichia coli* and recently *Listeria monocytogenes* pose a major health threat and warrant intensive research and continued surveillance[1]. The majority of human infection of *Salmonella* is related to the ingestion of contaminated foods such as poultry, beef, pork, egg, milk, cheese, seafood, fruits, juices and vegetables[2]. A global estimation by WHO indicated that in 2005, nearly 1.8 million people died from diarrhoeal diseases primarily due to the consumption

of contaminated food and drinking water (www.who.int/mediacentre). Generally non-typhoidal Salmonellosis in humans usually is self-limiting confining to gastrointestinal tract, but when infection spreads beyond the intestine, or when immunocompromised persons are affected, it requires an appropriate therapeutic intervention with antibiotics[3]. In recent times, another major concern is the occurrence of multi-drug resistance among the *Salmonella* in food stuffs[4]. It is presumed that the extensive use of antibiotics, especially in livestock production, may have resulted in the increasing incidence of antibiotic resistance in food borne *Salmonella*[5]. Occurrence of such widespread multi-drug resistance among an array of bacterial pathogens, presents a deep predicament in the choice of drugs.

This study was conducted to determine the prevalence of *Salmonella* in the curries and to characterize the antibiotic sensitivity of the isolates. In addition, this study focused on the evaluation of plant extracts against food borne *Salmonella* showing drug resistance. Curry was chosen as the source material since this preparation is widely used

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Foundation Project: Supported by Research Laboratory for Biotechnology, Department of Biotechnology, AIMST University, Malaysia.

along with rice in most of the Asian countries. Besides, to the best of our knowledge no much investigations have been directed toward food borne infections of *Salmonella* involving curry samples.

2. Materials and methods

2.1. Sample collection

A total of 50 curry samples were collected from 18 different food stalls in Sungai Petani, Kedah Darul Aman, Malaysia. The samples were not collected in pre-sterilized plastic bags; instead, all the samples were collected using the plastic bags provided by those restaurants and stalls. This method was adopted with the objective of including any possible contamination during handling and packaging. Then, these samples were immediately brought to the laboratory for microbiological analysis.

2.2. Isolation of *Salmonella*

Each sample was thoroughly mixed and inoculated onto the *Salmonella*–Shigella (SS) agar using sterile swabs and then incubated at 35 °C for 48 h. The presumptive *Salmonella* colonies were then sub-cultured by streaking onto the fresh SS agar using a sterile inoculating loop and incubated for 48 h at 35 °C.

2.3. Identification of the *Salmonella* isolates

Since the selective media was used for the isolation, the presumptive *Salmonella* isolates were identified by two confirmatory biochemical tests, triple-sugar-iron (TSI) agar test and the urease test. The presumptive *Salmonella* colonies were directly stabbed into the TSI agar slant. The inoculated samples were incubated with loosened caps for 24 h at 35 °C. For the urease test, 2 loopful of pure and well isolated *Salmonella* colonies were inoculated into the urea broth. The inoculated tubes were shaken gently and incubated with loosened caps for 48 h at 35 °C in an incubator. The TSI agar was checked for the production of hydrogen sulphide (H₂S) gas and the alkalinity, while the urease test was checked for the degradation of urea in urea broth[6]. The *Salmonella* colonies that were hydrogen sulphide gas positive on TSI agar, urease negative were sub-cultured onto fresh SS agar plates. Then a single colony was transferred into 20 mL of nutrient broth (enrichment media) and incubated for 18 h at 35 °C for further studies.

2.4. Antimicrobial susceptibility testing

The isolates were tested for susceptibility to ampicillin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), kanamycin (30 µg) and penicillin (10 U) on Mueller–Hinton agar plates by the disc diffusion method. Overnight cultures, grown on nutrient broth (cultures were adjusted to 0.5 McFarland units), were spread evenly on Mueller–Hinton agar. The respective antibiotics discs were placed on the culture plates. The plates were incubated at 35 °C for 24 h and inhibition zones were measured. The sensitivity and resistance of the isolates towards the antibiotics were

determined as per the criteria of the National Committee for Clinical Laboratory Standards (NCCLS)[7].

2.5. Minimum inhibitory concentration (MIC)

Selected *Salmonella* isolates which were sensitive to different antibiotics, subjected to MIC studies by the broth dilution method. For each antibiotic, a stock solution (100 µg/mL) was prepared and used for the serial dilution in nutrient broth (NB). The *Salmonella* isolates were grown in NB and incubated at 35 °C for 18 h. This cell suspension (OD adjusted to 0.5 McFarland units) was added to NB tubes serially diluted with antibiotics. The tubes were incubated at 35 °C for 24 h and observed for the turbidity.

2.6. Preparation of plant extracts

The dried and powdered leaves of *Camelia sinensis* (Theaceae) (tea leaves) and the *Trachyspermum ammi* (Apiaceae) (ajwain or omum seeds) powdered seed were used to prepare the aqueous extracts. Approximately 250 g of the plants materials were added to 650 mL boiling double distilled water in a 2 L beaker and allowed to infuse for 5 h separately. The extracts were decanted, filtered and concentrated in a rotary evaporator.

The isolates were tested for its susceptibility towards both the plant extracts using the disc diffusion method on the Mueller Hinton agar. *Salmonella* isolates grown in nutrient broth at 35 °C for 12 h (cultures were adjusted to 0.5 McFarland units), were spread evenly on Mueller–Hinton agar using sterile cotton swabs. Sterile 6 mm Whatman filter paper discs were impregnated with 20 µL of the plant extracts, which were dissolved in dimethyl sulfoxide (DMSO). The possible inhibitory effect on DMSO was also evaluated by placing sterile discs impregnated with the solvent. The plates were kept at room temperature to facilitate the diffusion of the extracts into the agar containing the isolate. Subsequently, the plates were incubated at 35 °C for 24 h. The plates were observed for the inhibition zones after the incubation and the zone of inhibition was recorded in mm.

2.8. Statistical analysis

All the experiments were conducted in triplicate. The data were recorded as Mean±SEM.

3. Results

Out of fifty curry samples collected from 18 different food outlets only 7 (14%) samples were contaminated with *Salmonella* by appropriate microbiological methods and biochemical tests. The presumptive *Salmonella* isolates were confirmed by biochemical tests such as triple sugar iron agar test and urease test. The presumptive colonies were scored to be *Salmonella* if the colonies showed positive reaction to triple sugar iron test and negative to urease test (Figure 1, 2). The pure colonies displayed typical *Salmonella* morphological characteristics on *Salmonella*–Shigella agar, which were clearly with a black spot in the center due to H₂S gas production.



Figure 1. The presumptive *Salmonella* colony after culturing from curry sample.

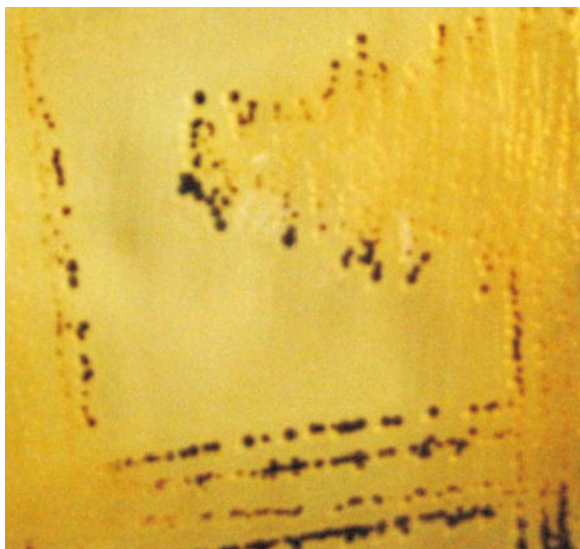


Figure 2. The pure *Salmonella* colonies obtained after sub-culturing from the presumptive colony on the fresh SS agar.

The drug sensitivity assay of the *Salmonella* isolates against the commercial antibiotics revealed that all (100%) of the isolates were resistant to ampicillin, tetracycline and chloramphenicol (Figure 3). However, all the isolates except isolates 29 and 46 showed minimal inhibition zones for the tetracycline but the obtained results are still regarded as resistant based on the NCCLS standard. In the case of penicillin, only 2 isolates (28.6%) were resistant, whereas, the rest of the isolates were either susceptible or intermediate. With reference to kanamycin, all the isolates were susceptible to this antibiotic (Figure 4). Besides, 71.4% of the isolates showed the multiple-drug-resistance (MDR) to three antibiotics, while, the rest 28.6% of the isolates exhibited MDR to four antibiotics.

Despite the sensitivity of the *Salmonella* isolates to penicillin and kanamycin, their sensitivity to these antibiotics varied. The MIC studies indicated that, for penicillin the MIC is 6.25 $\mu\text{g/mL}$ for the isolates 14, 27, 29, 30, while 3.13 $\mu\text{g/mL}$ for the isolate 34. With reference to kanamycin, the MIC is 12.50 $\mu\text{g/mL}$ for the isolates 14, 29 and 30. However, the isolates 27, 34, 41 and 46 required a higher concentration (25.00 $\mu\text{g/mL}$) for their growth inhibition.

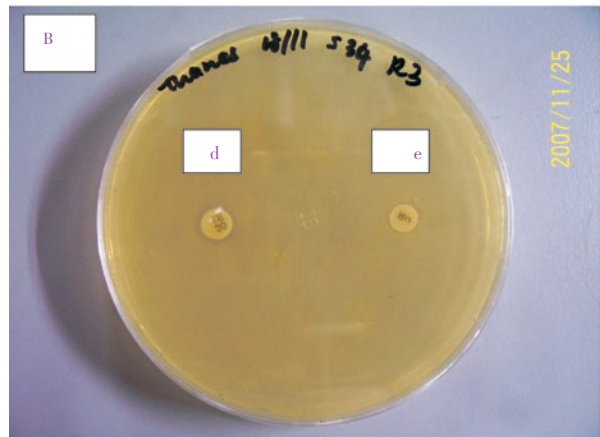


Figure 3. Antimicrobial susceptibility testing using commercial antibiotics.
a: Kanamycin; b: Ampicillin; c: Penicillin; d: Tetracycline; e: Chloramphenicol.

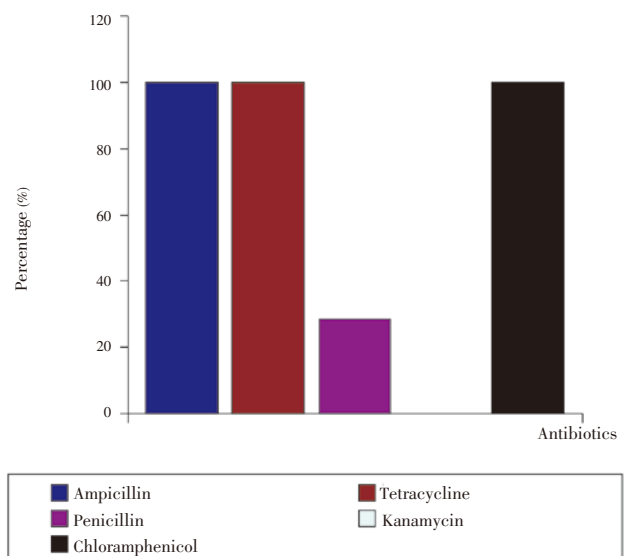


Figure 4. The resistance pattern of *Salmonella* isolates towards the commercial antibiotics.

The studies on the antimicrobial potential of the aqueous leaf and seed extracts of *Camelia sinensis* and *Trachyspermum ammi*, respectively indicated antibacterial property toward the *Salmonella* isolates. *Trachyspermum ammi* seed extract showed a better result in terms of the inhibition zones compared with the tea leaves extract (Figure 5). Based on this result, both two extracts can be used as herbal protectants to prevent or inhibit the growth of food borne *Salmonella*.

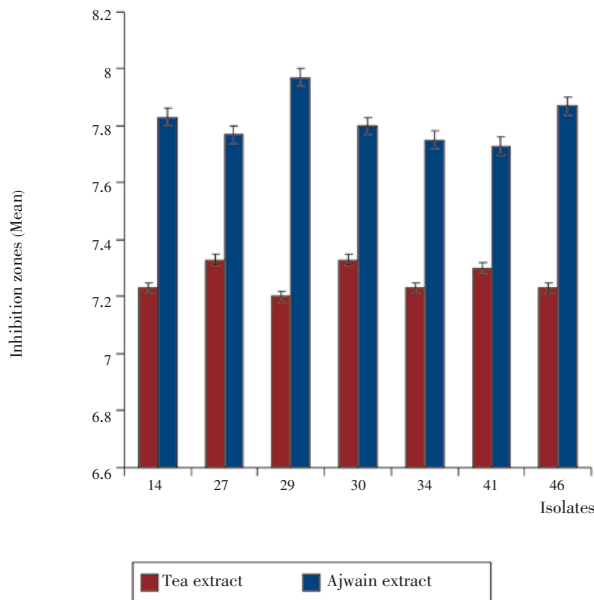


Figure 5. The antimicrobial effect of the plant extracts against *Salmonella* isolates.

4. Discussion

Diarrhoeal diseases are major causes of morbidity and mortality especially among children in developing countries after the malaria and respiratory infections^[8]. In Asia, Africa and Latin America, it has been estimated that 2.5 million deaths due to diarrhoea occur each year in children below 5 years of age^[9]. It has been well established that pathogens and other bacteria are transmitted from animals to humans through the food chain^[10]. Contamination of these foods can occur at multiple steps along the food chain, including production, processing, distribution, retail marketing, and handling or preparation^[11]. Since *Salmonella* has been isolated invariably from a majority of food items including vegetables, the contamination at various stages of food processing and handling has been considered as the major factor contributing to Salmonellosis among humans.

Despite considerable attention has been directed to eradicate typhoid, *Salmonella* food poisoning continued to be a leading public health problem worldwide. Since most *Salmonella* infections are acquired from ingestion of contaminated foods of animal origin, a likely cause for the increasing prevalence of antimicrobial-resistant *Salmonella* is the use of antimicrobial agents in food animals^[12].

The present study demonstrates the occurrence of *Salmonella* in the curry samples, which invariably forms a part of daily intake by people particularly in Asian countries. Probably, this report may be one among very few investigations to indicate the *Salmonella* contamination in curry samples. This study has also confirmed the prevalence of a varying drug resistance pattern among the *Salmonella* isolates. This may be due to the presence of more than one serovar of *Salmonella* in the curry samples. Increasing antibiotic resistance can limit the therapeutic options available to physicians for clinical cases that require antibiotic treatment. Therefore, there is a need to find strategies to minimize the risk of spreading antimicrobial resistance among animal and human populations.

In traditional folk medicine, tea decoction is considered to relieve gastritis to some extent especially in self-limiting infections. Similarly, *Trachyspermum ammi* seed is more

popular among the Indian families all over the world as the seeds have been used as food additives over centuries. In Indian folk medicine, the decoction prepared with this seeds has been traditionally used to relieve gastritis and diarrhea. The aqueous extraction was preferred as these plant materials are consumed in the form of tea decoction and as a food additive incorporated during cooking. This study has also revealed the antimicrobial properties of these plants materials against the isolates. And detailed pharmacological studies on these plants will reveal the potential use as antimicrobial compounds.

In general, proper food handling and hygienic food preparation practices should be followed. A continued surveillance on the outbreaks of food poisoning would limit the occurrence and spread of Salmonellosis among the population through environmental contamination. Inclusion of medicinal herbs in the food preparation especially in those involving meats of farm animals may minimize the possible contamination of food borne pathogens.

Conflict of interest statement

We declare that we have no conflict of interest.

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