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The prevalence of multidrug-resistant *Salmonella* in raw shrimp and octopus in Campeche, México

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ABSTRACT

Introduction: Multidrug-resistant (MDR) pathogenic bacteria in seafood, especially shrimp and octopus, are significant public health concerns and are able to be transmitted to humans in foodstuffs, particularly when they are of animal origin. The present study was conducted to measure the antimicrobial susceptibility patterns and prevalence of *Salmonella* isolates obtained from octopus and shrimp in San Francisco de Campeche, Mexico.

Methods: Two hundred shrimp and octopus samples (one hundred each) were collected from the municipal market, and each sample consisted of 100 g. The present study used conventional methods to identify and isolate *Salmonella*, with the disk-diffusion method used to screen all isolates for sensitivity to 12 antibiotics.

Results: The prevalence of *Salmonella* was found to be 56% and 45% in shrimp and octopus, respectively. The high levels of *Salmonella* observed in the municipal market sampled by the present study reveal poor sanitary conditions in the processing and transport of the products of interest and those handling them at the point of sale. All the *Salmonella* strains were resistant to at least three antimicrobial classes. All shrimp isolates (100%) presented susceptibility to chloramphenicol and the majority (88%) presented sensitivity to ciprofloxacin, while the strains isolated in the octopus individuals sampled presented sensitivity to both the foregoing antibiotics (74% and 90%, respectively).

Conclusion: Based on the high prevalence in the samples analyzed, our results suggest that shrimp and octopus could be involved in *Salmonella* infections in the population.

Keywords: Antibiotics; bacteria; foodborne; pathogen; seafood

INTRODUCTION

One of the most commonly observed foodborne pathogens on a global level (1), *Salmonella* mainly causes mild-to-severe gastroenteritis, which sometimes develops into potentially serious infection, such as bacteremia and endovascular infections, and even death (2). This pathogen is estimated to account for 80.3 million incidences of foodborne illness (3), with approximately 1.2 million cases, 23,000 hospitalizations, and 450 deaths (4) estimated to occur in the United States of America (USA) each year, while *Salmonella* typhimurium and *Salmonella* enteritidis are the most commonly identified serotypes (5). *Salmonella* spp. and *Salmonella* typhi are endemic in Mexico, where 452,861 and 189,274 cases of

salmonellosis and typhoid fever, respectively, were reported from 2015 to 2019 (6). The high prevalence of *Salmonella* represents a significant burden on both public health and the public purse in numerous countries around the world (7).

Eggs and poultry are the main sources of *Salmonella* spp. in developed and developing countries. As *Salmonella* has been identified in raw Mexican beef, chicken, and pork (8-11), meats with the high levels of consumption in Mexico, it represents a severe risk to public health. In addition, the occurrence of *Salmonella* spp. is reported to be higher in Asian and African seafood than in such products from America and Europe, with shrimp a common carrier (12,13). On the Mexican coast, the shrimp resource is one of the most important exportable marine products (14). Ending in March, the 2019–2020 seasonal harvest in Mexico produced 47 thousand 664 tons of shrimp (15). In the state of Campeche, in 2017, 4,870.91 tons were produced, ranking 6th nationwide (16). The Mexican octopus is another sea product associated with high quality, nutritional

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value, regulated production, and sound marketing systems, which places Mexico as the third-largest distributor of this abundant species in the world. In 2018, 14934.10 tons of octopus were caught and processed in Campeche (17). This seafood is among the most consumed on the coasts of Mexico. However, due to poor hygiene practices employed during processing and subsequent potential for contamination, on their consumption, infections caused by antibiotic-resistant *Salmonella* strains are reported in Vietnam, Iran, and India (17-19).

As this pathogen is able to acquire antibiotic resistance determinants, *Salmonella* isolates are often resistant to many different classes of antimicrobial agents. The presence of multidrug-resistant (MDR) pathogenic bacteria in seafood, especially shrimp and octopus, is a cause for significant concern in terms of public health. The transmission of MDR pathogens to humans commonly occurs through the consumption of food, especially products of animal origin (20). Foodborne illness are traditionally associated with seafood because pathogens are easily transmitted through cultivation, handling, and processing (21). There are no data currently available describing the presence of MDR *Salmonella* strains in raw shrimp and octopus in Mexico. Therefore, the present study aimed to determine the prevalence of MDR *Salmonella* in raw shrimp and octopus obtained at a municipal market in Campeche, Mexico. This study will provide helpful information for assessing the possible risk to consumers posed by this pathogen, which has a significant impact on public health in Mexico.

METHODS

One hundred raw shrimp and 100 raw octopus samples were collected randomly, for 6 months (March–August), from the public markets of San Francisco de Campeche, a city in the state of Campeche, Mexico. Ten samples were analyzed weekly (one per retailer), and each sample consisted of 100 g. Once collected in a sterile plastic bag, the samples were immediately transported to the laboratory in an icebox for microbiological analysis. The presence of *Salmonella* was determined in accordance with the method described in the Bacteriological Analytical Manual published by the United States Food and Drug Administration (22).

900 mL of buffered peptone water was added to each sample (100 g) and then homogenized in a stomacher (Stomacher 400 Circulator, Seward, UK) for 2 minutes, followed by incubation at 37°C for 24 hours. Then, 0.1 mL of the pre-enriched broth was added to 10 mL of Rappaport Vassiliadis broth (Merck, Mexico) (22), while a further 1 mL was inoculated into 10 mL tetrathionate broth (Bioxon, Mexico), with both incubated at 42°C for 24 h. The enrichment samples were streaked into brilliant green sulfa agar (Difco, Mexico), XLD agar (Bioxon, Mexico) and BS agar (Bioxon, Mexico) plates and then incubated for 24–48 h at 37°C. Two typical *Salmonella* strians per plate were inoculated in TSI agar and LIA agar and incubated at 35°C for 24 h (22), with the isolated bacteria then tested for urease production. Agglutination tests were done with *Salmonella* polyvalent O and H antisera procured from the Institute of Epidemiological Diagnosis

and Reference, which falls under the auspices of the Federal Secretariat of Health. Streaked on Tryptic Soy Agar slants, the *Salmonella* isolates were then kept at 3-5°C.

The antimicrobial susceptibility of the *Salmonella* isolates was ascertained for 12 antimicrobial agents through the Minimum Inhibitory Concentration method (23). First, each *Salmonella* strain was tested for susceptibility to antimicrobials on Mueller-Hilton Agar (Bioxon Mexico) plate, after which disks with impregnated antibiotics were used: Trimethoprim/sulfamethoxazole 1.25/23.75 μg/mL, ampicillin ten μg/mL, sulfisoxazole 250 μg/mL, chloramphenicol 30 μg/mL, cephalothin 30 μg/mL, tetracycline 30 μg/mL, gentamicin 10 μg/mL, amikacin 30 μg/mL, ceftriaxone 30 μg/mL, carbenicillin 100 μg/mL, ciprofloxacin 5 μg/mL, and erythromycin 15 μg/mL. All the antibiotics used were procured from Sigma Chemical (USA). After incubation for 24 h at 37°C, the growth inhibition zone around each disk was measured and classified as susceptible, resistant, and intermediary based on Clinical and Laboratory Standards Institute criteria (24).

RESULTS

A lower prevalence was identified for *Salmonella* in the octopus samples (45%) examined by the present study than that identified in the shrimp samples (55%), which coincides with the high *Salmonella* frequency (56.7%, 59.6%, and 49.1%) reported in shrimp in other investigations (19-25).

We isolated 226 *Salmonella* strains from the raw shrimp and octopus, and all the strains were resistant to a minimum of three classes of antimicrobial agents. One hundred and ninety strains isolated from shrimp and octopus (resistance of 89% and 80%) exhibited resistance to ampicillin (Tables 1 and 2). In addition, tetracycline and erythromycin showed a high resistance following cefalotin.

Gentamicin and ceftriaxone showed the same intermediate resistance (35%) (Table 1). The strains isolated from octopus exhibited the same resistance to gentamicin and streptomycin (44%).

All our isolated strains from shrimp presented a susceptibility to chloramphenicol, while the majority presented sensitivity to ciprofloxacin and the strains isolated from the octopus samples presented sensitivity to ciprofloxacin (90%).

DISCUSSION

This high prevalence of *Salmonella* in shrimp and octopus (56 and 45%, respectively) may result from contamination during production, and poor hygiene practices during handling, storage, and points of distribution and sale, also may be due to poor product temperature control as demand is very high, and cross-contamination is thought negligible by producers and consumers alike. However, the consumer may be unaware of the low levels of *Salmonella* needed for infection, suggesting better public understanding of the risks involved could allow for informed choices (market purchasing time etc. morning vs. evening). In 2008, Indian samples from clams and shrimp demonstrated prevalence of 34.4% and 26.7%, and in Malaysia, a prevalence of 37.5% was reported (26,27). In Vietnam observed a 24.5%

TABLE 1. Number (N) and percentage (%) of *Salmonella* strains isolated from shrimp

Antibiotic	R [N(%)]	I [N(%)]	S [N(%)]
Ampicillin	101 (89)	5 (5)	7 (6)
Tetracycline	80 (71)	5 (4)	28 (25)
Erythromycin	74 (65)	20 (18)	19 (17)
Carbenicillin	70 (62)	10 (9)	33 (29)
Cefalotin	61 (54)	25 (22)	27 (24)
Amikacin	57 (50)	24 (21)	32 (28)
Gentamicin	57 (50)	40 (35)	16 (14)
Trimethoprim/Sulfamethoxazole	43 (38)	36 (32)	34 (30)
Ceftriaxone	30 (26)	40 (35)	43 (38)
Sulfisoxazole	25 (22)	38 (34)	50 (44)
Ciprofloxacin	10 (9)	3 (3)	100 (88)
Chloramphenicol	0 (0)	0 (0)	113 (100)

Resistance (R), intermediate resistance (I) or sensitivity (S).

TABLE 2. Number (N) and percentage (%) of *Salmonella* strains isolated from octopus

Antibiotic	R [N(%)]	I [N(%)]	S [N(%)]
Ampicillin	90 (80)	8 (7)	15 (13)
Erythromycin	75 (66)	3 (3)	35 (31)
Tetracycline	68 (60)	35 (31)	10 (9)
Cefalotin	67 (59)	25 (22)	21 (19)
Carbenicillin	65 (58)	28 (25)	20 (18)
Streptomycin	50 (44)	30 (27)	33 (29)
Gentamicin	50 (44)	25 (22)	38 (34)
Trimethoprim/Sulfamethoxazole	48 (42)	30 (27)	35 (31)
Ceftriaxone	40 (35)	30 (27)	43 (38)
Sulfisoxazole	30 (27)	15 (13)	68 (60)
Chloramphenicol	20 (18)	9 (8)	84 (74)
Ciprofloxacin	5 (4)	7 (6)	101 (90)

Resistance (R), intermediate resistance (I) or sensitivity (S)

frequency of *Salmonella* (28). In 2015, researchers found that *Salmonella*'s prevalence was 22% also in shrimp samples (17). On the other hand, the results of those studies are higher than those reported in Venezuela, where a 2% frequency for raw shrimp was reported (29). In addition, other studies have also reported prevalence rates of 1.8% a 5.71% in shrimp (29,30-32), which are much lower than our results (64.1%).

A higher prevalence of *Salmonella* was observed in the octopus samples than that reported by a previous study conducted in Campeche in 2016; they exhibited a prevalence of 11.7% 63 (540) for this pathogen isolated from octopus (33), while in India, a prevalence of 16.6% was recorded (26). These different prevalence levels may occur as a result of socioeconomic factors (34).

Various studies have found that the presence of *Salmonella* in seafood occurs through exposure in either contaminated coastal waters or the unhygienic environments in which catches are landed and handled (35).

The high incidence of this microorganism in Campeche Bay may be associated with the discharges of domestic wastewater directly connected to the sea (36).

The high prevalence of positive *Salmonella* species in shrimp and octopus causes substantial public health risks, while the high prevalence observed in the municipal market by the present study is an indicator of poor sanitary conditions during processing and the poor hygiene of those handling the products at the point of sale. Typically reaching the aquatic environment through fecal contamination, *Salmonella* is thought to be able to survive for months or even years post-fecal shedding (13,37).

High resistance to ampicillin in this study (84.5%) agrees with other studies showing resistance to ampicillin of 100% (38,39). This suggests that medication with this antibiotic only generates economic expense with little or no benefit. In contrast, in Morocco, only 28 (49.1%) *Salmonella* strains were resistant to ampicillin (40).

In another study in Saudi Arabia in 2014, 140 *Salmonella* strains were extracted from different types of fish, with most isolates presenting resistance to tetracycline (90.7%) (41), a finding that contrasts with that obtained by the present study for *Salmonella* strains extracted from shrimp and octopus (71% and 60%, respectively). In contrast, in China showed *Salmonella* serovars extracted from seafood presented resistance to tetracycline (35.9%) (42). Furthermore, in Thailand, *Salmonella* strains isolated from shrimp sold in open markets were resistant to tetracycline (43).

The results obtained here for streptomycin (50% and 44% resistance) coincide with those reported by a study which found that *Salmonella* strains extracted from African catfish were sensitive to the same antibiotic (43.5%) (44). Similarly, in Nigeria, *Salmonella* serovars from ready-to-eat shrimp 65% (13/20) were resistant to streptomycin. They also observed that 100% of the strains isolated presented resistance to erythromycin, while we observed 65% in shrimp and 66% in octopus (45).

While Streptomycin is not a treatment for *Salmonella* infection, it is sometimes used to promote growth in some species, meaning that it could be used as a marker for resistant isolates circulating in the environment (46).

Salmonella isolated from Chinese fish ponds was resistant to erythromycin (47). Likewise, in Sri Lanka, *Salmonella* resistance to erythromycin was detected in shrimp (48). Our results exhibited 65% and 66% resistance to erythromycin. Chromosomally-located resistance determinants may mediate resistance, which may also occur through the acquisition of resistance genes by means of horizontal transfer through plasmids and transposons (49). The resistance to ceftriaxone was 35%. Such levels are concerning because of this antibiotic's importance in treating *Salmonella* in children. Its resistance is due to the AmpC β-lactamase (bla_{CMY-2}) gene (50).

The results obtained by the present study coincide with those reported by studies conducted in Thailand and Vietnam, wherein *Salmonella* strains isolated from vegetables and shrimp presented sensitivity to chloramphenicol (32% and 92% respectively) (51,52), while we observed 95%.

In Mexico, chloramphenicol-resistant strains have been extracted from such foodstuffs as pork, chicken, and vegetables (9,53). In addition, Miranda et al. mentioned that the presence of floR and cmlA genes may be associated with chloramphenicol resistance (10).

Resistance to sulfamethoxazole/trimethoprim was widespread in the *Salmonella* strains isolated from shrimp and octopus (38% and 42%); this finding agrees with studies conducted in various countries such as China (54-56).

These results are not unexpected, given that trimethoprim has been used with sulfamethoxazole for 30 years in both human and veterinary medicine (57). The presence of integron-borne dihydrofolate reductases represents the primary mechanism of trimethoprim resistance (8). Thirteen *Salmonella* strains isolated from shrimp and five isolated from octopus showed resistance to ciprofloxacin. In addition, ampicillin-resistant strains that are sensitive to ciprofloxacin have been isolated in salads comprising raw vegetables (58). Likewise, other researchers have observed *Salmonella* strains that are susceptible to ciprofloxacin (30). In addition, sensitivity to ciprofloxacin has been reported in strains extracted from cow manure and human feces in Ethiopia and Nigeria, which results from the fact that ciprofloxacin is barely used in these countries (39).

The results observed for ciprofloxacin by the present study concur with those obtained by research conducted on *Salmonella* strains extracted from chicken and beef and which also presented sensitivity to the antibiotic.

Due to its broad-spectrum activity, ciprofloxacin, a fluoroquinolone compound, is a good antibiotic used to treat *Salmonella* infections (59). However, the increased prevalence of antimicrobial-resistant *Salmonella* may result in more limited therapeutic options when a clinical case requires antimicrobial treatment (60). Antibiotic resistance is a complex and evolving challenge, given the emergence of resistant bacteria in various sectors, such as agriculture and human and veterinary medicine.

The resistance to different antimicrobials observed in *Salmonella* by the present study might be due to the bacteria being exposed, for long periods, to waters into which loads of antibiotics used for human and veterinary consumption and plant diseases are regularly discharged, since there is no wastewater treatment plant in Campeche.

The results obtained by the present study show that raw shrimp and octopus sold in the municipal market of San Francisco de Campeche commonly countain *Salmonella* strains resistant to multiple antibiotics, which poses a serious risk to public health in the region. Raw shrimp and octopus are not consumed in Mexico, but shrimp marinated in lemon is regularly consumed. In 2016, researchers demonstrated that *Salmonella* was able to survive in the concentrations of fresh lime juice that are typically used in the preparation of ceviche (30); this can be a risk for consumers, which can be limited by cooking.

CONCLUSION

Major resistance patterns found were mutiresistant strains. The most effective antibiotics against *Salmonella* in this study location were ciprofloxacin, chloramphenicol, and sulfisoxazole. Therefore, the monitoring of both antibiotic use in the general population and the hygiene standards observed in the processing and subsequent handling of seafood are imperative to avoid the acquisition and transmission of MDR *Salmonella*.

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CONFLICT OF INTEREST STATEMENT

The authors have declared no conflicting of interests.

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