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
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 strains 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, sulfoxazole 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%
The high prevalence of positive Salmonella strains isolated from octopus samples than that reported by a previous study conducted in various countries such as China (54-56). 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 isolated 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 (blaCMX) 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).

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

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

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

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

Resistance (R), intermediate resistance (I) or sensitivity (S).
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 salps 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 contain 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 may be limited by cooking.

CONCLUSION

Major resistance patterns found were multiresistant 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.

ACKNOWLEDGMENTS

The authors express their thanks to PRODEP for the financial support provided to this study. Apoyo a la incorporación de NPTC.

REFERENCES


