Antibiotic profile of *Vibrio parahaemolyticus* isolated from Sardine in Tripoli, Libya

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نبذة وصورة عن المضادات الحيوية لبكتريا الضمة المحللة للدم المعزولة من أسماك السردين في طرابلس، ليبيا

المستخلص:

تشكل المقاومة المتزايدة المضادة الحيوية الناجمة عن مسببات الأمراض المنقولة عن طريق الأغذية، مثل البكتيريا الهوائية والبكتيريا غير الهوائية، مشكلة عالمية هامة في مجال الصحة العامة. وقد أدى الإفراط في استخدام المضادات الحيوية في البشر والزراعة والتربية المائية إلى تلوث مياه المجارير والمياه المستعملة ومياه الأنهار، مما أثر على المناطق البحرية الساحلية ومزارع تربية الأحياء المائية. وتصنف الأدوية المضادة للميكروبات إلى فئتين: العوامل المضادة للميكروبات التي تحدث بصورة طبيعية وتستمد من الكائنات الحية، والأدوية الاصطناعية المضادة للبكتيريا. وأصبح ظهور المقاومة المضادة للأحياء في البكتريا الضمة المحللة للدم يشكل تهديداً كبيراً لصناعة التربية المائية. وخلصت الدراسة إلى أنها أظهرت أعلى مستوى من المقاومة للامبيسيلين (90%)، يليه الأموكسيسيلين – حمض الكالفيولانيك (63.3 %)، وسيفوتيكسيم (50%)، وسيفيبيم (50%)، وسيفتازيديم (46.7%)، وتيتراسيلكين (36.6%)، وأميكاسين (26.7%). وأظهرت غالبية العزلات معدل تعرض قدره 93.4% للميروبينم، و سيبروفلوكساسين ، وليفوفلوكساسين، واوفلوكساسين. وحددت الدراسة أيضاً مستوى معتدلاً من مقاومة الأميكاسين وتيتراسيكلين. وقد أظهرت جميع العزلات أنها غير مقاومة للإمبينيم (100%)، والجينتاميسين والبيبيراسيلين (96.7%)، والميروبينيم وللكوينولون (سيبروفلوكساسين، وليفوفلوكساسين، واوفلوكساسين) (%). ويكتسي مؤشر تعدد مقاومة المضادات الحيوية، وهو مقياس لتقييم المخاطر الصحية، أهمية حاسمة في تقييم قابلية العقاقير للتعرض لها ورصد ملامح المقاومة المضادة للميكروبات. وتسلط الدراسة الضوء على الحاجة إلى زيادة الوعي بالبكتريا الضمة المحللة للدم وعلاجها، التي يمكن أن تؤدي إلى التهاب الجهاز الهضمي.

الكلمات المفتاحية: *بكتريا الضمة المحللة للدم، المقاومة المضادة للأحياء الحيوية، الرقم القياسي (مؤشر تعدد مقاومة المضادات الحيوية).*

Abstract:

The increasing antibiotic resistance caused by foodborne pathogens, such as aerobic and anaerobic bacteria, is a significant global public health problem. The overuse of antibiotics in humans, agriculture, and aquaculture has led to contamination of sewage, wastewater, and river water, affecting coastal sea areas and aquaculture farms. Antimicrobial medicines are classified into two categories: naturally occurring antimicrobial agents derived from living organisms and synthetic antibacterial medicines. The emergence of antibiotic resistance in *V. parahaemolyticus* has become a significant threat to the aquaculture industry. This study uses the disc diffusion method to assess and enhance the antibiotic susceptibility of *V. parahaemolyticus* isolates. The results show

that the antibiotic susceptibility of the isolates was significantly improved after using the disc diffusion method. The study conducted antibiotic susceptibility testing on *V. parahaemolyticus* from shrimp found that they exhibited the highest level of resistance to ampicillin (90%), followed by amoxicillin-clavulanic acid (63.3%), cefotaxime (50%), cefepime (50%), ceftazidime (46.7%), tetracycline (36.6%), and amikacin (26.7%). The majority of the isolates exhibited a susceptibility rate of 93.4% to meropenem, ciprofloxacin, levofloxacin, and ofloxacin. The study also identified a moderate level of resistance to amikacin and tetracycline. All *V. parahaemolyticus* isolates exhibited susceptibility to imipenem (100%), gentamicin and piperacillin (96.7%), and meropenem and quinolone antibiotics (ciprofloxacin, levofloxacin, and ofloxacin) (93%). The MAR index, a measure for assessing health risk, is crucial in assessing the susceptibility of drugs and monitoring the profile of antimicrobial resistance. The study highlights the need for increased awareness and treatment for *V. parahaemolyticus* infections, which can lead to gastroenteritis.

Keywords: *Vibrio parahaemolyticus, Antibiotic resistance, MAR index*

Introduction

Following Alexander Fleming's 1928 discovery of penicillin, microorganisms have evolved defensive mechanisms against antibiotics and are constantly creating new resistance in order to survive. In 1937, sulfonamides were first used as potent antibacterial agents; however, reports of sulfonamide resistance date back to the late 1930s (Davies & Davies, 2010). Since the 1960s, there has been a scarcity of newly discovered antibiotic medications chemically modified to effectively cure infectious diseases, eradicate microorganisms, and minimize side effects. Since the 1970s, the production of antibiotic therapy has involved the amalgamation of medicines with distinct modes of action in order to enhance their efficacy. The excessive abuse of antibiotics in humans, agriculture, and aquaculture, as well as their use for controlling bacterial infections, has led to the global public health problem of antibiotic resistance. This problem arises due to the contamination of sewage, wastewater, and river water. It immediately impacts the coastal sea area and aquaculture farms (Okoh & Igbinosa, 2010; Radu *et al*., 2002; Silvester *et al*., 2015; Thakur *et al*., 2003).

Antimicrobial medicines are classified into two categories based on their origin. The first category comprises naturally occurring antimicrobial agents derived from living organisms, such as cephalosporin and penicillin, which are derived from fungi. The second category is the chemical kind, which refers to a synthetic antibacterial medicine (Riaz *et al*., 2011). Antimicrobial medications have different modes of action, including decreasing cell wall formation, cell membrane function, protein synthesis, and nucleic acid synthesis (Harris, 1964). The growing antibiotic resistance of foodborne pathogens, such as aerobic and anaerobic bacteria, is currently the most significant food safety problem (Devi *et al*., 2009a; Yong *et al*., 2003). The overuse of antibiotics is exacerbating the issue of multidrug resistance, where bacteria develop resistance to many antibiotics (Letchumanan *et al*., 2015; Livermore, 2003). Furthermore, the lack of comprehensive documentation about antibiotic-resistant bacteria in aquaculture is evident (Cabello, 2006). Symptoms of gastroenteritis, including diarrhea, vomiting, headache, fever, and, in severe cases, bloody diarrhea, frequently link to foodborne illness. Severe gastroenteritis caused by *V. parahaemolyticus* is typically treated with antibiotics such as tetracycline, ciprofloxacin, and cephalosporin (ceftazidime) as alternative treatments, although the condition generally resolves on its own (Devi et al., 2009b; Saifedden et al., 2016a). In the past, *Vibrio* has been sensitive to all antibiotics, except for the observed ampicillin resistance in *V. parahaemolyticus* and *V. vulnificus*. In recent decades, there has been a growing concern about the safety of food due to the increasing problem of antimicrobial resistance. Specifically, the emergence of antibiotic resistance in *V. parahaemolyticus* has become a significant threat to the aquaculture industry (Han *et al*., 2007; Tendencia & de la Peña, 2001**)**. The occurrence of *V. parahaemolyticus*, which is resistant to many drugs, is relatively high in Southeast Asia (Devi et al., 2009). In this article, we utilize the disc diffusion method (Bauer *et al*., 1966) to assess and enhance the antibiotic susceptibility of *V. parahaemolyticus* isolates.

Material and Method:

Sampling:

A total of 45 samples of Sardine fish spices were acquired at a local wet market situated in Tripoli Port, Libya. The samples were transferred to sterile plastic bags for transit and subsequently processed and tested on the same day.

Bacterial isolates:

Shrimp samples bought in Tripoli, Libya, yielded a total of 30 *V. parahaemolyticus* isolates. A 10-gram part of each sample was placed in a sterile stomacher bag and mixed with 90 milliliters of tryptic soya broth (TSB) from BactoTM, France, containing 3% NaCl from Merck, Germany. The mixture was then churned in a blender for one minute. Next, the sample was cultured on thiosulfate-citrate-bile salts-sucrose agar (TCBS) from LIOFILCHEM, Italy. Incubate the culture at 37 °C for 18–24 hours. The *V. parahaemolyticus* colonies manifest a green hue on the TCBS agar, and the colonies were subsequently transferred to a new TCBS agar medium for purification utilizing a sterile toothpick.

Antibiotic susceptibility:

The antibiotic susceptibilities of 30 isolates were assessed using the disk diffusion method, as suggested by CLSI (Clsi, 2006), as described by (Bauer et al., 1966). After direct immersion in a 4 mL solution of normal saline inoculum containing 0.85% NaCl, equivalent to the 0.5 McFarland standard, the colony was evenly applied onto a Mueller-Hinton (MH) agar plate (Merck, Germany) using a sterile cotton swab. The inoculum was evenly applied onto a Mueller-Hinton (MH) agar plate (Merck, Germany) using a sterile cotton swab. After applying the inoculum onto a Mueller-Hinton (MH) agar plate using a sterile cotton swab, it was air-dried for 3-5 minutes at room temperature. Before placing the antibiotic discs on the MH agar, the plates were subjected to overnight incubation at a temperature of 37°C. Afterward, the size of the area where growth was inhibited was measured, and the findings were analyzed according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI, 2010) M45-2A.

Fourteen antibiotic disks from Oxoid, UK, were utilized in this study. The specific antibiotics and their respective dosages were as follows: ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), piperacillin (100 µg), imipenem (10 µg), meropenem (10 µg), amikacin (30 µg), gentamicin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), ofloxacin (5 µg), cefepime (30 µg), cefotaxime (30 µg), and ceftazidime (30 µg). These antibiotics were chosen based on the recommendations provided by the Clinical and Laboratory Standards Institute (CLSI) in their M45-2A guidelines published in 2010.

The multiple antibiotic resistance index (MAR)

The MAR index is a valuable instrument that offers a precise evaluation of the source of contamination (Krumperman, 1983). The MAR index is determined by dividing the number of antibiotics that show resistance by the total number of antibiotics that the isolates are exposed to (Elexson *et al*., 2014; Osundiya *et al*., 2013).

Results:

Antibiotic susceptibility testing was conducted on *V. parahaemolyticus*, which was obtained from shrimp. Fourteen antibiotics were chosen from various categories for this purpose. According to Figure 1, *V. parahaemolyticus* isolates exhibited the highest level of resistance to ampicillin (90%), followed by amoxicillin-clavulanic acid (63.3%), cefotaxime (50%), cefepime (50%), ceftazidime (46.7%), tetracycline (36.6%), and amikacin (26.7%). Around 6% of the drugs, including meropenem, ciprofloxacin, levofloxacin, and ofloxacin, exhibited a minor level of resistance. Nevertheless, the isolates exhibited a high susceptibility rate of 100% to imipenem, as well as 96.7% susceptibility to piperacillin and gentamicin. The majority of the isolates exhibited a susceptibility rate of 93.4% to meropenem, ciprofloxacin, levofloxacin, and ofloxacin. Furthermore, the *Vibrio* isolates exhibited a moderate susceptibility to amikacin, tetracycline, ceftazidime, and amoxicillin-clavulanic acid, with susceptibility rates of 73.3%, 63.3%, 53.3%, and 36.7%, respectively. The MAR index was derived by dividing the number of antibiotic-resistant bacteria by the total number of bacteria tested. A MAR index greater than 0.2 was seen in almost half of the *V. parahaemolyticus* isolates, which means they were not sensitive to at least one antibiotic. The specified minimum acceptable rate (MAR) ranged from 0.07 to 0.71. The isolate (Vp.19) exhibited the highest MAR index, indicating resistance to 10 antibiotics.

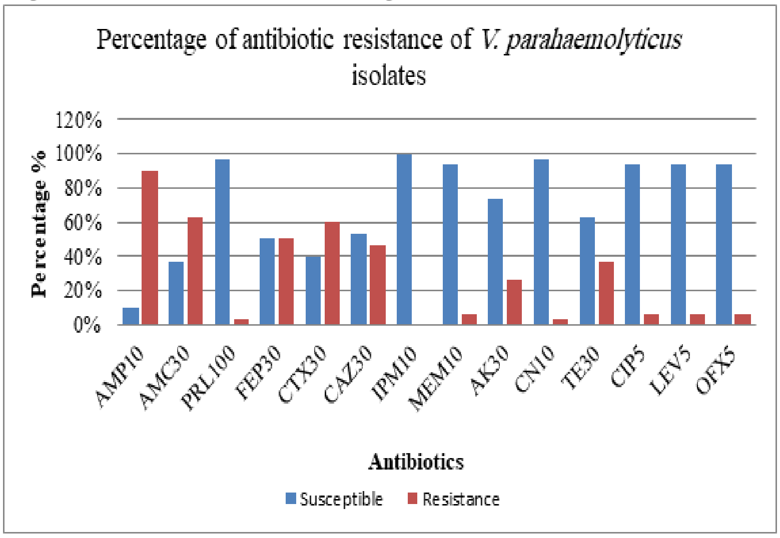

Figure1. Percentage of antibiotic susceptibility result.

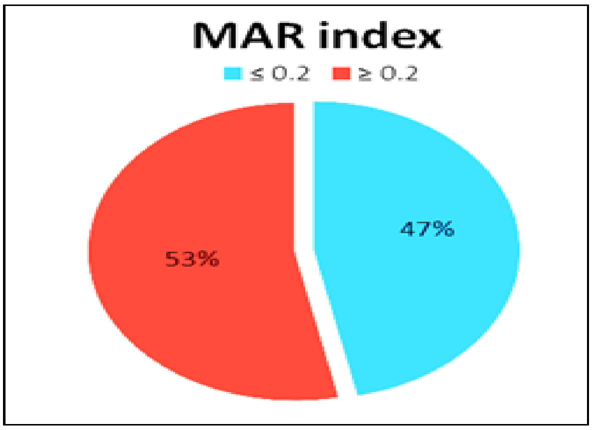

Figure 2. MAR index chart shows percentage of low and high risk.

Discussion:

The consumption of infected seafood leads to gastroenteritis, a condition caused by *Vibrio parahaemolyticus*, a pathogen (Di Pinto *et al*., 2008). As per the Centers for Disease Control and Prevention, treatment for most cases of *V. parahaemolyticus* infection is unnecessary. Patients should consume ample fluids to prevent dehydration resulting from diarrhea. Antibiotics such as tetracycline and ciprofloxacin may be used in more severe instances, considering the antimicrobial susceptibilities of the organisms (Center for Disease Control and Prevention, 2006). The *V. parahaemolyticus* isolates in this investigation exhibited a 90% resistance rate to ampicillin, which aligns with findings from previous studies. *V. parahaemolyticus* isolates exhibited a high level of resistance to ampicillin, ranging from 80% to 90% (Al-othrubi *et al*., 2014; Han *et al*., 2007; Shaw *et al*., 2014; Yu *et al*., 2016).

Studies conducted since 1978 have consistently found that *Vibrio* bacteria have shown resistance to ampicillin, with a range of 40% to 90% (Sudha *et al*., 2014). This observation indicates that ampicillin has a low efficacy in treating *V. parahaemolyticus* infection. In addition, *V. parahaemolyticus* isolates exhibited a 50% resistance rate to AMC. Nevertheless, alternative research findings demonstrated a susceptibility range of 94% to 99% (Jiang *et al*., 2014; Saifedden *et al*., 2016b; Shaw *et al*., 2014), along with a relatively low resistance rate of approximately 6% (Yu *et al*., 2016). The paucity of data regarding the application of AMC to *V. parahaemolyticus* prevents accurate comparison with other studies. The isolates exhibited resistance rates of 46.7%, 60%, and 50% to third- and fourth-generation cephalosporin antibiotics (ceftazidime, cefotaxime, and cefepime), respectively. The outcome is comparatively higher than the resistance averages reported in another research, which ranged from 3% to 46% (Elmahdi *et al*., 2016; Letchumanan *et al*., 2015; Ubong *et al*., 2011; You *et al*., 2016; Zavala-Norzagaray *et al*., 2015). According to Jun et al. (2012), between 70% and 80% of *V. parahaemolyticus* isolates found in Korea in 2012 were not susceptible to cefotaxime or ceftazidime. On the other hand, (Saifedden *et al*., 2016) and Yu *et al*. (2016) suggested that cephalosporin could be a potential therapeutic option for Vibrio infections due to *V. parahaemolyticus*'s significant vulnerability to this antibiotic. The variations in the literature concerning the susceptibility of *V. parahaemolyticus* to cefotaxime may be attributed to geographical disparities. Furthermore, the classification of cefepime as a fourth-generation cephalosporin raises considerable concerns, even with its very modest

resistance rate. However, the current investigation identified a moderate level of resistance to amikacin and tetracycline, with rates of 26.7% and 36.6%, respectively.
All *V. parahaemolyticus* isolates exhibited susceptibility to imipenem (100%), gentamicin and piperacillin (96.7%), and meropenem and quinolone antibiotics (ciprofloxacin, levofloxacin, and ofloxacin) (93%). Previous research has shown that quinolone and tetracycline antibiotics can kill *V. parahaemolyticus* (Han et al., 2007; Shaw et al., 2014; Sudha et al., 2014; Yu et al., 2016). These results back this up. *Vibrio parahaemolyticus* also exhibited a notable susceptibility to imipenem, as reported by (Noorlis *et al*., 2011; Letchumanan *et al*., 2015). The development of antibacterial resistance is a complex process. Numerous researchers have argued that we must decrease the use of antimicrobial agents and improve their utilization for infection management. However, reducing the use of antimicrobials is not an option. This might be because the bacteria have already become resistant through the transfer of genetic elements (Livermore, 2003) . Liu *et al*. (2013) reported the presence of a new plasmid in a strain of *V. parahaemolyticus*. This plasmid has genes that confer resistance to several drugs. It is possible that these genes can be transferred to other *Vibrio* species by genetic elements. This process accelerates the development of multidrug resistance in *Vibrio*. Hence, it is imperative to consider all aspects that contribute to the escalation of bacterial resistance. The MAR index, which has a scale of 0 to 1.0, is a useful measure for assessing health risk. The MAR index value of 0.20 distinguishes between low and high risks. If the MAR value is greater than 0.20, then the sample is considered to have a high risk of source contamination (Krumperman, 1983). Paul *et al*. (1997) reported that the MAR index provides evidence of all isolates originating from an environment with excessive antibiotic use. Researchers regard the MAR index as a reliable technique for assessing risk. However, there are contradictory results on antibiotic susceptibility and multiple antibiotic resistance (MAR) from different geographical regions. The *V. parahaemolyticus* isolates obtained from Tripoli, Libya, exhibited a significantly high prevalence of multiple antibiotic resistance (MAR), reaching approximately 96%. These findings indicate that antibiotics from both human and animal sources may impact and pollute the aquatic environment in the sampling area. The MAR (Multiple Antibiotic Resistance) assertion plays a crucial role in assessing the susceptibility of drugs and monitoring the profile of antimicrobial resistance. It enhances and guarantees the safety of food and public health.

Conclusion:

The consumption of infected seafood can lead to gastroenteritis, a condition caused by Vibrio parahaemolyticus, a pathogen. Treatment for most cases of V. parahaemolyticus infection is unnecessary, and patients should consume ample fluids to prevent dehydration. Antibiotics such as tetracycline and ciprofloxacin may be used in more severe cases. The isolates in this investigation exhibited a 90% resistance rate to ampicillin, indicating that ampicillin has a low efficacy in treating V. The isolates also exhibited resistance rates of 46.7%, 60%, and 50% to third- and fourth-generation cephalosporin antibiotics (ceftazidime, cefotaxime, and cefepime), respectively. The current investigation identified a moderate level of resistance to amikacin and tetracycline, with rates of 26.7% and 36.6%, respectively. All V. parahaemolyticus isolates exhibited susceptibility to imipenem (100%), gentamicin and piperacillin

(96.7%), and meropenem and quinolone antibiotics (ciprofloxacin, levofloxacin, and ofloxacin) (93%). The development of antibacterial resistance is a complex process, and reducing the use of antimicrobial agents is not an option. The Multiple Antibiotic Resistance (MAR) assertion plays a crucial role in assessing drug susceptibility and monitoring the profile of antimicrobial resistance, enhancing and guaranteeing the safety of food and public health.

Recommendations:

Further research is required in the field of aquaculture and water analysis to identify the origins of antibiotic contamination. Further research should be conducted on *V. parahaemolyticus* to enhance public health. The assessment of antibiotic resistance is essential for identifying any alterations that may aid in prescribing appropriate medicine for infections.

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