

ANTIBACTERIAL ACTIVITY OF COCONUT SHELL LIQUID SMOKE AGAINST *Vibrio parahaemolyticus* CAUSING AHPND IN WHITELEG SHRIMP (*Litopenaeus vannamei*)

Aktivitas Antibakteri Asap Cair Tempurung Kelapa Terhadap Bakteri *Vibrio parahaemolyticus* Penyebab *Acute Hepatopancreatic Necrosis Disease* (AHPND) pada Udang Vaname (*Litopenaeus vannamei*)

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ABSTRACT

Acute hepatopancreatic necrosis disease (AHPND), caused by *Vibrio parahaemolyticus*, is a major bacterial disease that significantly impacts *Litopenaeus vannamei* aquaculture. The use of natural antibacterial agents has gained increasing attention as an alternative to antibiotics. This study aimed to evaluate the antibacterial activity of coconut shell liquid smoke against *V. parahaemolyticus* using an in vitro disc diffusion method. A completely randomized design was applied with five concentrations of liquid smoke (0.5, 1.0, 1.5, 2.0, and 2.5%), along with positive (chloramphenicol) and negative (distilled water) controls, each with three replications. Antibacterial activity was assessed by measuring the diameter of inhibition zones after 24 hours of incubation. The results showed that coconut shell liquid smoke exhibited significant antibacterial activity against *V. parahaemolyticus*. The highest inhibition zone was observed at 1.0% concentration (18.03 mm), followed by 0.5% (13.63 mm), both categorized as strong inhibition. However, the antibacterial effect did not increase proportionally with concentration, indicating a non-linear response. The positive control showed a very strong inhibition zone (26.5 mm), while no inhibition was observed in the negative control. In conclusion, coconut shell liquid smoke demonstrates strong potential as a natural antibacterial agent against *V. parahaemolyticus*. Further in vivo studies are recommended to evaluate its effectiveness in shrimp culture systems for sustainable AHPND management.

Keywords: AHPND, Antibacterial Activity, Coconut Shell Liquid Smoke, *Litopenaeus vannamei*, *Vibrio parahaemolyticus*

ABSTRAK

Acute Hepatopancreatic Necrosis Disease (AHPND) yang disebabkan oleh *Vibrio parahaemolyticus* merupakan salah satu penyakit bakterial utama yang menyebabkan kerugian signifikan dalam budidaya udang vaname (*Litopenaeus vannamei*). Penggunaan antibakteri

berbasis bahan alami menjadi alternatif strategis untuk mengurangi ketergantungan terhadap antibiotik sintetis. Penelitian ini bertujuan untuk mengevaluasi aktivitas antibakteri asap cair tempurung kelapa terhadap *V. parahaemolyticus* secara in vitro menggunakan metode difusi cakram. Penelitian disusun menggunakan rancangan acak lengkap dengan lima taraf konsentrasi asap cair (0,5; 1,0; 1,5; 2,0; dan 2,5%), serta kontrol positif (kloramfenikol) dan kontrol negatif (akuades), masing-masing dengan tiga ulangan. Aktivitas antibakteri ditentukan berdasarkan diameter zona hambat yang terbentuk setelah inkubasi selama 24 jam. Hasil penelitian menunjukkan bahwa asap cair tempurung kelapa memiliki aktivitas antibakteri terhadap *V. parahaemolyticus*. Zona hambat tertinggi diperoleh pada konsentrasi 1,0% sebesar 18,03 mm, diikuti oleh konsentrasi 0,5% sebesar 13,63 mm, yang keduanya dikategorikan sebagai daya hambat kuat. Peningkatan konsentrasi tidak menunjukkan hubungan linier terhadap diameter zona hambat, yang mengindikasikan adanya interaksi kompleks senyawa aktif dalam asap cair. Kontrol positif menunjukkan daya hambat sangat kuat dengan diameter zona hambat sebesar 26,5 mm, sedangkan kontrol negatif tidak menunjukkan adanya aktivitas penghambatan. Dengan demikian, asap cair tempurung kelapa berpotensi sebagai agen antibakteri alami terhadap *V. parahaemolyticus*. Penelitian lanjutan secara in vivo diperlukan untuk mengevaluasi efektivitas aplikatifnya dalam sistem budidaya udang sebagai strategi pengendalian AHPND yang berkelanjutan.

Kata Kunci: Asap Cair Tempurung Kelapa, *Vibrio parahaemolyticus*, Aktivitas Antibakteri, AHPND, *Litopenaeus vannamei*

INTRODUCTION

Shrimp aquaculture, particularly Pacific white shrimp (*Litopenaeus vannamei*), has experienced rapid growth and has become a major contributor to global aquaculture production (FAO, 2020). However, the increasing intensification of culture systems has heightened shrimp susceptibility to disease outbreaks, particularly those caused by pathogenic bacteria. One of the most destructive bacterial diseases in shrimp farming is Acute Hepatopancreatic Necrosis Disease (AHPND), primarily caused by *Vibrio parahaemolyticus* (Kumar et al., 2021). This disease is characterized by severe damage to the hepatopancreas and can lead to mass mortality, with reported mortality rates reaching up to 100% within a short period after infection (Anissa et al., 2023).

The occurrence of AHPND has resulted in significant economic losses in shrimp-producing countries, including Indonesia, due to reduced survival rates and production yields (Rizky et al., 2026). Conventional disease control strategies still largely rely on the use of antibiotics. However, the excessive and uncontrolled use of antibiotics has led to several critical issues, including the emergence of antimicrobial resistance, environmental contamination, and the presence of residues in aquaculture products (Soto-Rodriguez et al., 2022). These concerns highlight the urgent need to develop alternative disease control strategies that are environmentally friendly and sustainable.

In recent years, natural products have gained increasing attention as antibacterial agents in aquaculture. One promising candidate is liquid smoke derived from coconut shells. Liquid smoke is a product of lignocellulosic biomass pyrolysis and contains various bioactive compounds, including phenolic compounds, organic acids, and carbonyl compounds, which are known for their antimicrobial properties (Fitra et al., 2023; Muriady et al., 2022). These compounds have been reported to inhibit bacterial growth through mechanisms such as disruption of cell membranes, increased membrane permeability, and interference with enzymatic systems and bacterial genetic material (Swastawati et al., 2023).

Previous studies have demonstrated the potential of liquid smoke to inhibit the growth of various pathogenic bacteria. For instance, liquid smoke at different concentrations has been

reported to significantly suppress the growth of *Aeromonas hydrophila* (Priyantono and Kusdarwati, 2022). However, studies specifically evaluating the effectiveness of coconut shell liquid smoke against *V. parahaemolyticus*, the causative agent of AHPND in shrimp, remain limited and require further investigation.

Based on these considerations, this study aimed to evaluate the antibacterial activity of coconut shell liquid smoke against *V. parahaemolyticus* using an in vitro approach. The findings of this study are expected to provide a scientific basis for the application of liquid smoke as a natural antibacterial agent to support sustainable AHPND disease management in shrimp aquaculture.

RESEARCH METHODS

Experimental Design

This study employed an in vitro experimental approach to evaluate the antibacterial activity of coconut shell liquid smoke against *Vibrio parahaemolyticus*. Antibacterial activity was assessed using the disc diffusion (Kirby–Bauer) method, which is widely applied in preliminary antimicrobial screening due to its high reproducibility and ability to represent the diffusion capacity of antibacterial compounds in solid media (Muriady *et al.*, 2022). A completely randomized design (CRD) was applied with five concentrations of liquid smoke: 0.5%, 1.0%, 1.5%, 2.0%, and 2.5% (v/v). Chloramphenicol was used as a positive control, while sterile distilled water served as a negative control. Each treatment was conducted in triplicate.

Bacterial Isolate and Inoculum Standardization

The bacterial isolate used in this study was *Vibrio parahaemolyticus* obtained from a laboratory culture collection and maintained on Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar. Prior to testing, a single colony was inoculated into Nutrient Broth supplemented with 2–3% NaCl and incubated at 28–30°C for 18–24 h under shaking conditions (approximately 120 rpm) to obtain an uluñu culture. The bacterial suspension was standardized to 0.5 McFarland turbidity (approximately 1×10^8 CFU/mL), which is commonly used in antimicrobial susceptibility testing to ensure consistency. The suspension was subsequently diluted to a working concentration of approximately 10^6 CFU/mL before use.

Preparation of Liquid Smoke

Coconut shell liquid smoke was obtained commercially and used as the test substance. Serial dilutions were prepared aseptically using sterile distilled water to obtain concentrations of 0.5%, 1.0%, 1.5%, 2.0%, and 2.5% (v/v). Chloramphenicol (30 µg/disc) was used as the positive control due to its broad-spectrum activity against Gram-negative bacteria, including *Vibrio* spp., while sterile distilled water was used as the negative control to confirm the absence of antibacterial effects from the solvent.

Antibacterial Activity Assay (Disc Diffusion Method)

The antibacterial activity assay was performed using the disc diffusion (Kirby–Bauer) method with slight modifications (Muriady *et al.*, 2022). A volume of 100 µL of the standardized bacterial suspension was evenly spread onto the surface of TCBS agar using a sterile spreader to form a uniform bacterial lawn. Sterile paper discs (6 mm in diameter) were then loaded with approximately 20 µL of liquid smoke solution at each concentration and placed aseptically onto the inoculated agar surface. Positive and negative control discs were also placed on the same plate. All plates were incubated at 28–30°C for 24 h under aerobic conditions. The use of TCBS agar was intended to support the selective growth of *Vibrio* spp., thereby ensuring specificity of the assay.

Measurement and Interpretation of Inhibition Zones

Following incubation, antibacterial activity was determined by measuring the diameter of the inhibition zones (clear zones) formed around the discs (Fitra Adinda et al., 2023). Measurements were performed using a digital caliper with a precision of 0.01 mm. To improve accuracy, measurements were taken in two perpendicular directions and averaged. The inhibition strength was interpreted according to the classification of Stout and Davis (1971): weak (<5 mm), moderate (5–10 mm), strong (10–20 mm), and very strong (>20 mm).

Data Analysis

Inhibition zone data were expressed as mean \pm standard deviation. Prior to statistical analysis, normality and homogeneity of variance tests were conducted to verify data assumptions. Data were then analyzed using one-way analysis of variance (ANOVA) to determine the effect of liquid smoke concentration on inhibition zone diameter. When significant differences were observed ($p < 0.05$), Duncan's Multiple Range Test (DMRT) was applied for post hoc comparison among treatments. All analyses were performed using appropriate statistical software.

RESULT

Characteristics of *Vibrio parahaemolyticus* Isolate

Observations on selective Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar (Figure 1) showed that the bacterial isolate formed circular colonies with convex surfaces, exhibiting green to yellow coloration.

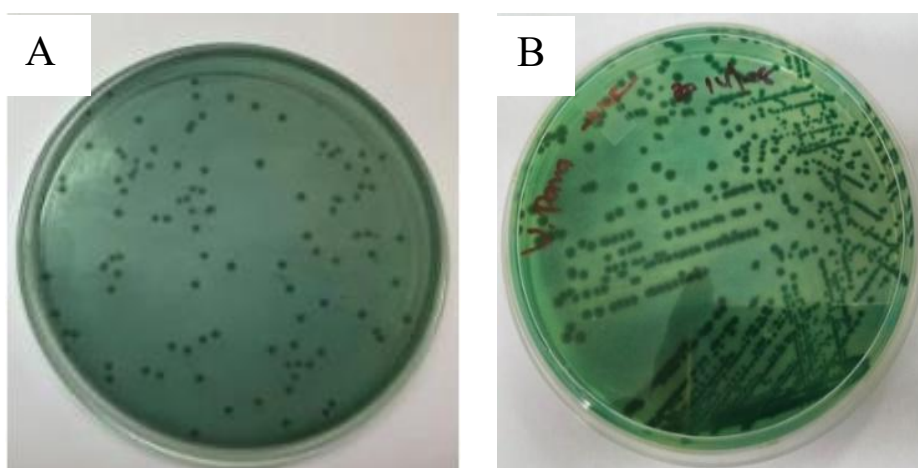


Figure 1. Morphological Characteristics of *V. parahaemolyticus* Colonies on TCBS Agar Showing Green and Yellow Colonies with Convex Surfaces

TCBS agar is selective due to the presence of bile salts and citrate, which inhibit the growth of non-*Vibrio* bacteria, thereby facilitating specific isolation of the target organism. These observations confirm that the isolate used in this study corresponds to *V. parahaemolyticus*, a known causative agent of AHPND.

Antibacterial Activity of Coconut Shell Liquid Smoke

The antibacterial assay results (Figure 2) demonstrated the formation of inhibition zones at all tested concentrations of coconut shell liquid smoke against *V. parahaemolyticus*. Each label (A–E) represents different concentrations, while (+) and (–) denote positive (chloramphenicol) and negative (distilled water) controls, respectively. The positive control

exhibited the largest inhibition zone, indicating high antibacterial effectiveness, whereas the negative control showed no inhibition, confirming the absence of solvent effects.

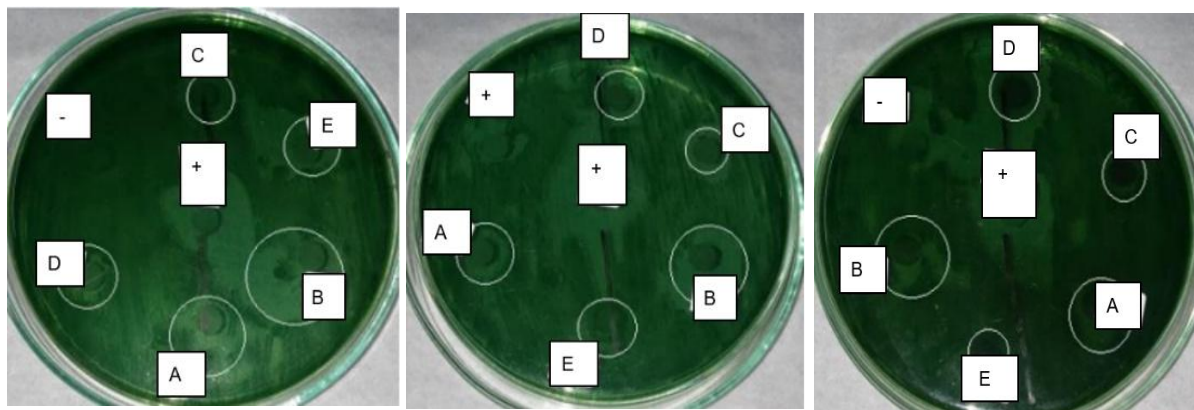


Figure 2. Inhibition Zones of Coconut Shell Liquid Smoke at Various Concentrations Against *V. parahaemolyticus*

Inhibition zones are formed due to the diffusion of active compounds from the disc into the agar medium, suppressing bacterial growth in the surrounding area. The largest inhibition zone was observed at 1.0% concentration (18.03 mm), followed by 0.5% (13.63 mm), both categorized as strong inhibition. In contrast, the 1.5% concentration showed a decrease in inhibition (8.80 mm, moderate category), followed by an increase at 2.0% and 2.5% (Table 1).

Table 1. Inhibition Zone Diameter of Coconut Shell Liquid Smoke Against *V. parahaemolyticus*

Treatment	Inhibition Average (mm)			Mean (mm)	Responses
	I	II	III		
Negative Control (-)	0	0	0	0	Weak
Chloramphenicol (+)	26.3	26.7	26.5	26.5	Very strong
0.5 % (A)	16.5	13.2	11.2	13.63	Strong
1 % (B)	19.8	15.6	18.7	18.03	Strong
1.5% (C)	8.7	8.9	8.8	8.8	Medium
2% (D)	10.7	15.6	13.15	13.15	Strong
2.5% (E)	11.6	12.6	10.55	11.56	Strong

Statistical Analysis and Variation in Antibacterial Response

Analysis of variance (ANOVA) revealed that liquid smoke concentration had a significant effect ($p < 0.05$) on inhibition zone diameter. Duncan's Multiple Range Test (DMRT) indicated that the 1.0% concentration exhibited significantly higher antibacterial activity compared to several other treatments. However, some treatments, particularly 0.5%, 2.0%, and 2.5%, did not show significant differences. This variation suggests that antibacterial response is not solely dependent on concentration but is also influenced by interactions among active compounds and experimental conditions.

DISCUSSION

Yellow colonies indicate sucrose fermentation resulting in acid production, whereas green colonies indicate non-sucrose-fermenting bacteria. These characteristics are consistent with the morphological description of *Vibrio parahaemolyticus*, which is capable of growing on TCBS agar and displaying colony color variation depending on sucrose metabolism (Soto-Rodriguez et al., 2022). The variation in colony color is associated with sucrose fermentation

ability: yellow colonies produce acid that alters the pH indicator in the medium, while green colonies do not ferment sucrose (Kumar et al., 2021). These characteristics indicate that the isolate is appropriate for antibacterial testing.

The variation in inhibition zone diameter across concentrations indicates that antibacterial activity does not increase linearly with concentration. This phenomenon may be attributed to the complex composition of liquid smoke, which contains phenols, organic acids, and carbonyl compounds (Fitra et al., 2023). These compounds may interact synergistically or antagonistically, influencing antibacterial effectiveness at specific concentrations (Muriady et al., 2022).

Additionally, the effectiveness of the disc diffusion method is strongly influenced by compound diffusion in agar media. Compounds with larger molecular size or higher viscosity tend to diffuse more slowly, resulting in smaller inhibition zones despite higher concentrations (Kowalska-Krochmal and Dudek-Wicher, 2021). This may explain the reduced inhibition observed at 1.5% concentration.

The positive control (chloramphenicol) exhibited the highest inhibition zone (26.5 mm), categorized as very strong, while the negative control showed no inhibition. These results confirm the reliability of the assay method in distinguishing active and inactive substances. Chloramphenicol inhibits bacterial protein synthesis by binding to the 50S ribosomal subunit, exerting bacteriostatic or bactericidal effects depending on concentration (Uddin et al., 2021). The strong effectiveness observed indicates that the tested bacteria remain sensitive to this antibiotic, validating its use as a reference.

According to Kowalska-Krochmal and Dudek-Wicher (2021), inhibition zone variability in disc diffusion assays can be affected by inoculum density, media composition, and compound diffusion rates. Therefore, interpretation of results should consider these factors comprehensively.

The antibacterial activity of coconut shell liquid smoke is primarily attributed to phenolic compounds, organic acids, and carbonyl compounds. Phenolic compounds disrupt bacterial cell membranes by increasing permeability and causing leakage of intracellular components. Organic acids reduce environmental pH and disrupt ionic balance within cells, while carbonyl compounds inactivate essential enzymes and proteins involved in bacterial metabolism (Swastawati et al., 2023). These mechanisms are particularly effective against Gram-negative bacteria such as *V. parahaemolyticus*, which possess an outer membrane susceptible to disruption by lipophilic compounds such as phenols (Dien et al., 2024). Thus, the presence of these bioactive compounds supports the potential of liquid smoke as a natural antibacterial agent.

The findings of this study demonstrate that coconut shell liquid smoke has potential as a natural antibacterial agent against *V. parahaemolyticus*, the primary causative agent of AHPND. The optimal concentration identified in this study was 1.0%, which exhibited the highest inhibition activity. These results align with the need for environmentally friendly disease control strategies, as discussed in the introduction. The use of natural compounds such as liquid smoke may reduce reliance on antibiotics and mitigate the risk of antimicrobial resistance (Navaneeth et al., 2020). However, since this study was conducted in vitro, further in vivo studies are required to evaluate its effectiveness, safety, and applicability in complex shrimp aquaculture systems.

CONCLUSION

Coconut shell liquid smoke demonstrated antibacterial activity against *Vibrio parahaemolyticus* under in vitro conditions, as evidenced by the formation of inhibition zones at all tested concentrations. The 1.0% concentration exhibited the highest inhibitory effect, with an inhibition zone diameter of 18.03 mm, categorized as strong, and can therefore be

considered the optimum concentration in this study. The non-linear antibacterial response observed across increasing concentrations indicates that the effectiveness of liquid smoke is influenced not only by concentration but also by the complex interactions among its bioactive compounds and their diffusion capacity in the medium. This suggests that concentration optimization is essential to achieve maximum antibacterial efficacy. Overall, coconut shell liquid smoke shows promising potential as a natural antibacterial agent for controlling *V. parahaemolyticus*, the causative agent of AHPND, in shrimp aquaculture (*Litopenaeus vannamei*). However, further *in vivo* studies are required to evaluate its effectiveness, safety, and applicability under more complex aquaculture conditions.

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REFERENCES

- Anissa, R. K., Lisdiana, L., & Widyayanti, A. T. (2023). Optimasi Metode Nested PCR untuk Deteksi *Vibrio parahaemolyticus* AHPND pada Udang Vaname (*Litopenaeus vannamei*), *LenteraBio: Berkala Ilmiah Biologi*, 13(1), 1–13. doi: 10.26740/lenterabio.v13n1.p1-13.
- Dien, H. A., Montolalu, R. I., Mentang, F., & Berhimpon, S. (2024). Stability of liquid smoked nano-encapsulated on the foodborne pathogens and histamine-forming bacteria's growth in tuna loin sashimi, *Nutricion Clinica y Dietetica Hospitalaria*, pp. 286–295. doi: 10.12873/443dien
- FAO. (2018). AHPND: Acute Hepatopacreatic Necrosis Disease. Asian Fisheries Society
- Fitra Adinda, R., Faisal, M. & Muhammad Djuned, F. (2023). Characteristics of Liquid Smoke From Young Coconut Shells at Various Pyrolysis Temperature, *Elkawnie*, 9(1), p. 24. doi: 10.22373/ekw.v9i1.14225.
- Kowalska-Krochmal, B. & Dudek-Wicher, R. (2021). The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. *Pathogens*, 10(2), pp. 1–21. doi: 10.3390/pathogens10020165.
- Kumar, V., Behera, B. K., Bossier, P., & Das, B. K. (2021). Acute hepatopancreatic necrosis disease (AHPND): Virulence, pathogenesis and mitigation strategies in Shrimp aquaculture. *Toxins*, 13(8), pp. 1–28. doi: 10.3390/toxins13080524.
- Muriady, Meilina, H. & Faisal, M. (2022). Antibacterial Activity of Liquid Smoke Powder From Rice Husk. *International Journal of GEOMATE*, 23(95), pp. 89–96. doi: 10.21660/2022.95.7522.
- Navaneeth, K. A., Bhuvaneswari, T., Rajan, J. J. S., Alavandi, S. V., Vijayan, K. K., & Otta, S. K. (2020). Characterization of *Vibrio parahaemolyticus* isolates from shrimp farms of Southeast coast of India with special reference to Acute Hepatopancreatic Necrosis Disease (AHPND) status. *Aquaculture*. Elsevier B.V, 518, p. 734813. doi: 10.1016/j.aquaculture.2019.734813.
- Prijantono, R. C. & Kusdarwati, R. (2022). The Effect of Coconut Shell Liquid Smoke (*Cocos nucifera*) Fish on Nile Tilapia Hematology. *Journal of Aquaculture Science*, 7(1), pp. 22–37.
- Rizky, P. N., Halim, A. M., Li'ani, A. S., & Cheng, T. C. (2026). Bactericidal and Immunostimulant Activity of *Scutellaria baicalensis* Georgi in Cobia (*Rachycentrol canadum* L.) Against *Photobacteriosis*. *Turkish Journal of Fisheries and Aquatic Sciences*, 26(1). doi: 10.4194/TRJFAS28014.

- Soto-Rodriguez, S. A., Lozana-Olvera, R., Montfort, G. R., Zenteno, E., Salgado, J. L., Vibanco, N., & Rendon, K. G. A. (2022). New Insights into the Mechanism of Action of PirAB from *Vibrio Parahaemolyticus*. *Toxins*, 14(4). doi: 10.3390/toxins14040243.
- Swastawati, F., Antami, H. A. & Purnamayati, L. (2023). Applying edible coating based on chitosan and liquid smoke on the quality and microstructure of barracuda fish sausage (*Sphyraena sp.*). *IOP Conference Series: Earth and Environmental Science*, 1137(1). doi: 10.1088/1755-1315/1137/1/012030.
- Uddin, T. M., Chakraborty, A. J., Khusro, A., Zidan, B. R. M., Mitra, S., Emran, T. B., Dhama, K., Ripon, K. H., Gajdacs, M., Sahibzada, M. U. K., Hossain, J., & Koirala, N. (2021). Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects', *Journal of Infection and Public Health*, 14(12), pp. 1750–1766. doi: 10.1016/j.jiph.2021.10.020.