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Case Study

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Bacteriological Investigation of Purulent Cutaneous Nodules in an Indo-Pacific Bottlenose Dolphin (*Tursiops aduncus*) at Samudra Ancol, Indonesia: A Case Study

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Background: The Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) is a marine mammal commonly found in Indonesian waters. However, it may develop health conditions, including purulent-cutaneous nodules, indicating bacterial infection.

Aim: This case study aimed to isolate bacteria from the cutaneous nodules in a bottlenose dolphin at Samudra Ancol, Indonesia, revealed antimicrobial activity, and use the result to support clinical treatment decisions.

Methods: A 26-year-old female dolphin presented with two purulent-cutaneous nodules (1 and 3 cm) on the ventrolateral abdomen. Nodule contents were collected using sterile swab following small skin incision and cultured on Nutrient Agar (NA), Mannitol Salt Agar (MSA), and Blood Agar (BA), followed by Gram staining and Triple Sugar Iron Agar (TSIA). *In vitro* antimicrobial activity was evaluated using diffusion assay. Rifampicin was administered for 3 months, accompanied by supportive supplementation, according institution protocol. Pool water was also examined for heterotrophic plate count (HPC) and total coliforms.

Results: The isolate exhibited Gram-positive cocci, growth with mannitol fermentation on MSA, and acid/acid reaction on TSIA, supporting its presumptive identification as *Staphylococcus* sp. Inhibition zones were observed in seven antibiotics. Following one month of treatment, the nodules decreased in size, with no visible pus or erythema. After three months, the nodules were no longer prominent, with minor residual skin changes. Pool water examination showed an HPC of 46 CFU/mL and no detectable coliform colonies.

Conclusion: The isolate was presumptively identified as *Staphylococcus* sp. and the dolphin showed clinical improvement following rifampicin treatment with supportive supplementation. Meanwhile, pool water microbiological quality met the facility's established criteria.

INTRODUCTION

The Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) is a marine mammal inhabiting coastal waters throughout the Indian Ocean and the western Pacific. This species is commonly associated with shallow coastal habitats and is classified as Near Threatened (NT) on the IUCN Red List, highlighting its vulnerability to increasing anthropogenic (human-induced) pressures in coastal environments (Haughey et al., 2021). As coastal habitats become increasingly exposed to human activities, understanding the health status of dolphin populations is important for effective wildlife health management and conservation.

Skin disorders are among the health problems reported in cetaceans (marine mammals, including dolphins, whales, and porpoises) and may arise from various infectious and non-infectious causes. Infectious agents associated with cetacean skin lesions include bacteria, viruses, and fungi, while non-

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infectious factors such as trauma, environmental conditions, and ectoparasites may also contribute to cutaneous abnormalities (Ewing et al., 2023; Gaydos et al., 2023; Segura-Göthlin et al., 2023). Cutaneous infections associated with *Staphylococcus* sp. have also been documented in dolphins. Notably, *Staphylococcus delphini* has been implicated in purulent skin lesions in dolphins, with subsequent reports confirming its role in cutaneous disease in bottlenose dolphins maintained under human care (Varaldo et al., 1988; Romani-Cremaschi et al., 2023). Other bacterial species, including *Staphylococcus aureus*, *Klebsiella* sp., and *Pseudomonas aeruginosa*, have also been isolated from cutaneous lesions in different captive bottlenose dolphins (Sánchez Martínez et al., 2015). These reports indicate that bacterial infections are among the potential causes of cutaneous lesions in dolphins and underscore the importance of bacteriological investigation in affected animals.

At Samudra Ancol, a marine mammal facility in North Jakarta, Indonesia, an Indo-Pacific bottlenose dolphin (Fig. 1A) was observed to have purulent cutaneous nodules. Considering the potential involvement of bacterial pathogens in these lesions, bacteriological investigation and antimicrobial susceptibility testing were conducted to support clinical management. Therefore, this study aimed to isolate and characterize bacteria recovered from the cutaneous nodules of an Indo-Pacific bottlenose dolphin, evaluate their antimicrobial susceptibility, and describe the therapeutic management based on the susceptibility test results.

Case Presentation

The case involved a 26-year-old female Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) housed at Samudra Ancol, North Jakarta, Indonesia (Fig. 1A). The dolphin was found to have cutaneous lesions on the ventral abdominal region, characterized by purulent nodules. At the time of clinical observation, the animal was generally active, with normal swimming activity, balanced swimming movements, and normal respiration. Its appetite was generally normal, although occasional decreases in food intake were observed. The skin lesions initially appeared as two nodules located on the ventrolateral abdominal region, measuring approximately 1 and 3 cm in diameter. Both nodules exhibited signs of inflammation, including erythema, an irregular surface, and the presence of purulent material surrounding the lesions (Fig. 1B).

MATERIALS AND METHODS

Animal Handling and Procedural Authorization

All procedures were performed in accordance with the institutional technical guidelines (Samudra Ancol) for swab sample collection (Document No. IK-KESWAN-19) and the corresponding medical procedure authorization letter (No. 01/KESWAN/04-2022). All sampling procedures followed the established institutional protocols and applicable regulations.

Nodule Swab Sample Collection

This study focused on the bacterial investigation of cutaneous nodules in an Indo-Pacific bottlenose dolphin



Figure. 1 Indo-Pacific bottlenose dolphin (*Tursiops aduncus*). (A) a 26-year-old female Indo-Pacific bottlenose dolphin. (B) Purulent nodules on the ventrolateral abdominal region of the dolphin, measuring 1 and 3 cm in diameter, with visible pus surrounding the lesions.

(*Tursiops aduncus*) housed at Samudra Ancol, North Jakarta, Indonesia. Samples were collected from a single female dolphin. A sterile-rayon swab with Amies agar gel without charcoal (Transystem™ Traditional Bacteriology Transport, 108C, California, USA) was used for sample collection and transportation. Swab samples were collected by making a small incision in the skin overlying the nodule using a sterile surgical blade. The contents of the nodule were then collected using a sterile-rayon swab (Fig. 2). After collection, the swab was placed back into the Amies agar gel without charcoal transport medium. The collected swab samples were immediately transported in a cool box to the Microbiology Laboratory at Samudra Ancol for bacterial isolation and subsequent characterization.

Bacterial Isolation and Phenotypic Characterization

All bacteriological procedures were performed aseptically. Before bacterial inoculation, Blood Agar (BA) was prepared by supplementing the agar base with 5-10% dolphin blood. The blood was collected from the periarterial venous rete (PAVR) in the ventral region of the fluke, following the established procedures at Samudra Ancol. The nodule swab samples were then inoculated onto Nutrient Agar (NA), Mannitol Salt Agar (MSA), and BA using the streak plate method. The inoculated media were then incubated at 37°C for 24 hours. Following incubation, colony morphology was examined macroscopically on all three media based on colony shape, elevation, margin, and colour. Uninoculated NA, MSA, BA, and TSIA media were incubated under the same conditions as negative controls to monitor potential contamination. Cellular morphology were also examined microscopically using the Gram staining method.

The bacterial growth was subsequently subjected to Triple Sugar Iron Agar (TSIA) assay. The TSIA test was performed according to the laboratory procedures established at Samudra Ancol. After incubated at 37°C for 24 hours, the TSIA reactions were then evaluated based on colour changes in the slant and butt of the medium.

***In Vitro* Antimicrobial Activity Assay**

The agar well diffusion assay was performed according to the laboratory procedures established at Samudra Ancol. Seven antimicrobial agents commonly used in aquatic animal medicine were selected based on their availability at the facility: enrofloxacin, rifampicin, sulfamethoxazole-trimethoprim, metronidazole, ciprofloxacin, amoxicillin-clavulanic acid, and ceftazidime.

Bacterial growth from the TSIA medium was collected using a sterile inoculating loop and suspended in 0.85% sodium chloride (NaCl) solution. The resulting bacterial suspension was evenly spread over the surface of NA using a sterile rayon swab. Wells were



Figure. 2 Sample collection of purulent material from a cutaneous nodule on the ventrolateral abdominal region of an Indo-Pacific bottlenose dolphin using a sterile-rayon swab following a small skin incision.

then aseptically made in the agar, and the respective antimicrobial preparations were loaded into the wells. Sufficient spacing was maintained between wells to prevent overlapping inhibition zones. The inoculated plates were incubated at 37°C for 18-24 hours, after which the inhibition zones surrounding the wells were examined.

The assay was conducted under the laboratory conditions and resources available at the facility. Given the need for timely clinical management of the affected dolphin, the agar well diffusion method was employed as a preliminary assessment of the *in vitro* inhibitory activity of the available antimicrobial preparations. The assay was intended to provide supportive information for clinical decision-making rather than to establish standardized antimicrobial susceptibility profiles.

Treatment and Clinical Monitoring

According to the institution protocol, the selected antibiotic and supplements were administered orally by placing the respective preparations into the gill cavities of mackerel or scad fish, which were subsequently offered to the dolphin as feed. Clinical changes in the cutaneous lesions were monitored throughout the treatment period.

Supporting Examination of Pool Water Samples

Pool water samples were collected and transferred into sterile bottles that had been previously sterilized and pre-filled with sodium thiosulfate to neutralize residual chlorine in the water samples. The bottles were then tightly sealed and wrapped in aluminium foil.

Total Heterotrophic Plate Count (HPC). The total heterotrophic bacterial count in the pool water samples was determined using the pour plate method on Marine Agar, a medium commonly used for the isolation, cultivation, and enumeration of heterotrophic bacteria in marine water. Briefly, 1 mL of each water sample was transferred into a sterile petri dish, followed by the addition of approximately 20 mL of Marine Agar. The sample and agar medium were gently mixed to ensure an even distribution and allowed to solidify. The plates were then incubated at 37°C for 18-24 hours. Following incubation, the number of bacterial colonies was counted to determine the total heterotrophic bacterial count.

Total Coliform Count. Total coliform bacteria in the pool water samples were enumerated using membrane filtration method with m-Endo medium. Briefly, the m-Endo medium was prepared by adding 2 mL of ethanol to 400 mL of distilled water, followed by homogenization using a magnetic stirrer. Approximately 2 mL of the prepared medium was dispensed into each petri dish. A 100-mL aliquot of the water sample was then filtered through a membrane filter. The membrane filter was subsequently placed onto the prepared medium in the petri dish and incubated at 37°C for 18-24 hours. Coliform bacteria were enumerated by counting colonies showing a characteristic metallic sheen on the medium, indicating the presence of coliform bacteria in the water samples (APHA et al., 2017).

RESULTS AND DISCUSSION

Bacterial Isolation and Phenotypic Characterization

Fig. 3 shows bacterial growth was observed on NA, MSA, and BA. On NA, the colonies appeared round, white, and convex (Fig. 3A). No bacterial growth was observed in any of the uninoculated control media (NA, MSA, BA, and TSIA) following incubation under the same conditions as the inoculated media, indicating the absence of detectable contamination in the negative control. NA is a general-purpose medium that supports the growth of a wide range of non-fastidious bacteria due to its nutrient content, including peptone and beef extract. Therefore, the observed growth confirmed the presence of culturable bacteria in the nodule sample, although the colony characteristics alone were not sufficient for bacterial identification. On MSA, bacterial growth was accompanied by a colour change in the medium from red to yellow (Fig. 3B), indicating mannitol fermentation. The colonies were round and convex, with entire margins. MSA is a selective and differential medium containing approximately 7.5% sodium chloride, that only support the growth of salt-tolerant bacteria, particularly members of the genus *Staphylococcus*. The yellow colour in media results from acid production during mannitol fermentation, which lowers the pH of the medium and changes the phenol red indicator from red to yellow. MSA is used to distinguish mannitol-fermenting from non-mannitol-fermenting Staphylococci (Thakur et al., 2017; ASM, 2024).

On BA prepared with 5-10% dolphin blood, the colonies appeared white, round, and smooth-edged, with no visible haemolysis (Fig. 3C). According to the laboratory procedure at Samudra Ancol, dolphin blood was used as a blood supplement for BA preparation.

The source of blood used in BA preparation is an important consideration, as previous studies have shown that blood from different animal species may influence bacterial growth and haemolytic patterns (Yeh et al., 2009; Egwuatu et al., 2014). In the present case, no clear or greenish zone was observed around the colonies, indicating that neither beta- nor alpha-haemolysis was detected under the test conditions.

Gram staining revealed purple-stained, coccus in single, in pairs, and in grape-like clusters (Fig. 3D). The purple-stained showed that the bacterial cells retained the crystal violet-iodine (CV-I) complex following decolorization, a characteristic of Gram-positive bacteria with a relatively thick peptidoglycan layer (Tille, 2017). The observed cellular morphology and arrangement were consistent with those typically described for *Staphylococcus* sp., which appear as Gram-positive cocci occurring in grape-like clusters (Schleifer & Bell, 2015). These result were supported by the MSA result, that bacterial growth was accompanied by a colour change from red to yellow, indicating mannitol fermentation. Taken together, the Gram-positive cocci morphology, cluster arrangement, and positive mannitol fermentation provide phenotypic evidence supporting the presumptive isolate identification as genus *Staphylococcus* (Tille, 2017; Kumari et al., 2020).

The TSIA test was performed as a supplementary biochemical examination to assess the carbohydrate fermentation profile of the bacterial isolate, following the established laboratory procedures at Samudra Ancol. The isolate exhibited an acid/acid

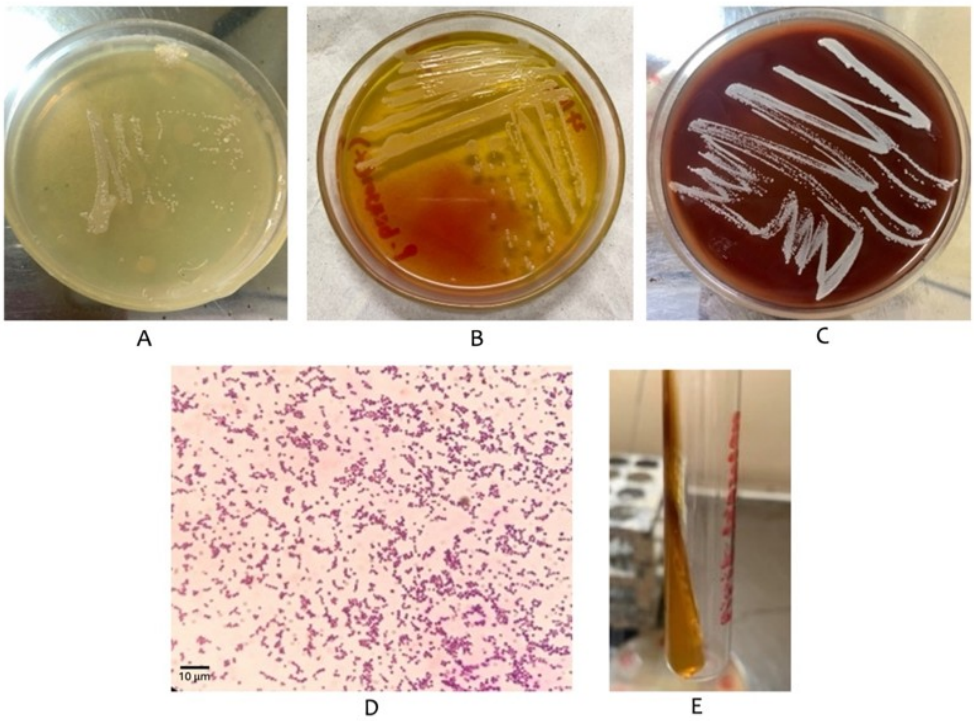


Figure. 3 Results of the bacteriological characterization of the bacterial isolate. (A) Growth on Nutrient Agar (NA), showing round, white, and convex colonies. (B) growth on Mannitol Salt Agar (MSA), accompanied by a colour change of the medium from red to yellow, indicating mannitol fermentation. (C) Gram staining, revealing purple-stained cocci arranged singly, in pairs, and in irregular grape-like clusters; (D) growth on Blood Agar (BA), showing white, round colonies with smooth margins and no visible haemolysis. (E) Triple Sugar Iron Agar (TSIA) reaction, showing an acid/acid (A/A) profile with yellow coloration in both the slant and butt, without gas production or black precipitate (Note: TSIA was performed as an additional biochemical examination in accordance with the established laboratory procedures at Samudra Ancol. The results were included to provide supplementary phenotypic information on the bacterial isolate).

(A/A) reaction, characterized by yellow coloration in both the slant and butt of the medium, with no detectable gas production or H_2S formation (Fig. 3E). This reaction indicates glucose fermentation accompanied by the fermentation of lactose and/or sucrose (ASM, 2016; Tille, 2017). However, TSIA is primarily used for the preliminary differentiation of enteric bacteria and is not a specific identification test for *Staphylococcus* sp. Therefore, the A/A reaction observed in this study is interpreted as an additional phenotypic characteristic of the isolate.

According to the phenotypic results, the isolate was presumptively identified as *Staphylococcus* sp. The clinical relevance of this genus in cetacean skin infections has been reported previously, including *S. delphini*, which was first isolated from purulent skin lesions in dolphins (Varaldo et al., 1988) and subsequently reported as a cause of cutaneous infection in a bottlenose dolphin under human care (Romani-Cremaschi et al., 2023). However, the present results do not confirm the isolate as *S. delphini* or any other specific species. Further biochemical and molecular analyses are required to confirm the identification.

In Vitro Antimicrobial Activity and Clinical Response to Treatment

Table. 1 presents the results of the *in vitro* antimicrobial activity assay conducted to evaluate the inhibitory effects of seven antimicrobial preparations against the bacterial isolate. Inhibition zones were observed for all seven preparations. Enrofloxacin produced the largest inhibition zone, measuring 36 mm, followed by ciprofloxacin (34 mm), metronidazole (31 mm), trimethoprim-sulfamethoxazole and amoxicillin-clavulanic acid (29 mm each), rifampicin (28 mm), and ceftazidime (27 mm) (Fig. 4 and Table. 1). These results showed that all tested antibiotics exhibited *in vitro* inhibitory activity against the isolate under the conditions of this assay. However, the inhibition zone diameters should not be interpreted as standardized susceptibility results or used to classify the isolate as susceptible or resistant.

Following the *in vitro* antimicrobial activity assay, rifampicin was selected by the attending veterinarian as the sole antibiotic for the treatment of the affected dolphin, based on clinical judgment. Although rifampicin did not produce the largest inhibition zone among the tested antimicrobial preparations, its selection was not determined solely by the *in vitro* inhibition results. Rifampicin is a semisynthetic rifamycin derivative with bactericidal activity that acts by inhibiting DNA-dependent RNA polymerase, thereby interfering with bacterial RNA synthesis. Rifampicin exhibits broad-spectrum antibacterial activity against several Gram-positive bacteria, including *Staphylococcus aureus* and other Staphylococcal species. It has also been used, primarily in combination with other antimicrobial agents, to manage various non-mycobacterial infections (Lee et al., 2017). Nevertheless, resistance to rifampicin can emerge rapidly, particularly when it is administered as monotherapy over a three-month period.

The clinical response was monitored throughout the three-month treatment period, as presented in Fig. 5. Before treatment, two prominent cutaneous

Table. 1 Diameter zone observed in *in vitro* antimicrobial activity assay

Antibiotic	Diameter (mm)	Result
Enrofloxacin	36	Inhibition observed
Rifampicin	28	Inhibition observed
Trimethoprim + sulfamethoxazole	29	Inhibition observed
Metronidazole	31	Inhibition observed
Ciprofloxacin	34	Inhibition observed
Amoxicillin + clavulanate acid	29	Inhibition observed
Ceftazidime	27	Inhibition observed

nodules with visible inflammatory changes were observed on the ventrolateral abdominal region (Fig. 5A). After one month of treatment, both nodules showed a reduction in size, although residual raised lesions were still visible (Fig. 5B). By the end of the three-month treatment period, the nodules were no longer visibly prominent, with only minor residual changes in the skin surface remaining (Fig. 5C). These results demonstrate progressive clinical improvement throughout the treatment period. However, the observed improvement cannot be attributed exclusively to rifampicin, as supportive supplementation was also provided. Furthermore, the disappearance of visibly prominent lesions does not necessarily confirm complete microbiological clearance of the infection. Although clinical improvement was observed during treatment, the use of rifampicin as monotherapy over a three-month period warrants consideration because of the risk of rapid resistance development in Staphylococci. Rifampicin monotherapy is generally discouraged for *Staphylococcus aureus* infections due to the potential emergence of resistance during treatment (Boyd et al., 2025).

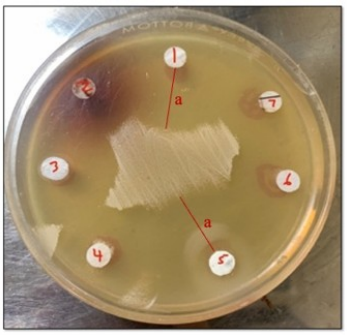


Figure. 4 *In vitro* antimicrobial activity assay showing the inhibition zones produced by different antimicrobial preparations against the bacterial isolate. The tested antimicrobial agents were (1) enrofloxacin, (2) rifampicin, (3) trimethoprim-sulfamethoxazole, (4) metronidazole, (5) ciprofloxacin, (6) amoxicillin-clavulanic acid, and (7) ceftazidime. The red lines labelled "a" means the radius of the inhibition zone; the inhibition zone diameter was calculated as twice the radius (diameter = 2 × a).

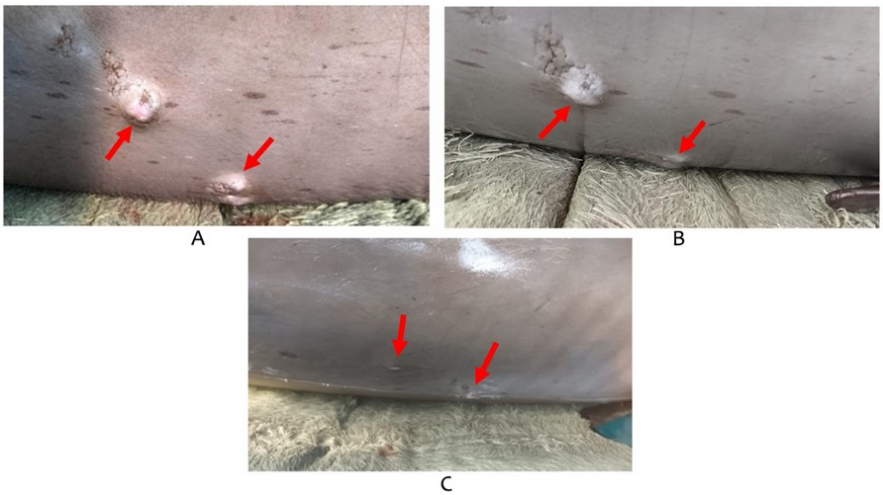


Figure. 5 Clinical progression of cutaneous nodules on the ventrolateral abdominal region of an Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) during rifampicin treatment. (A) Before treatment, showing two prominent nodules with inflammatory changes. (B) After one month of treatment, showing a reduction in nodule size. (C) After three months of treatment, showing the disappearance of visibly prominent nodules with minor residual skin changes.

Supporting Examination of Pool Water Quality

The supporting microbiological examination of the pool water revealed a HPC of 46 CFU/mL, which was below the internal bacterial colony threshold established by Samudra Ancol (<200 CFU/mL). Meanwhile, the total coliform examination showed no colonies with a characteristic metallic sheen on the m-Endo medium, representing a negative result for detectable coliform bacteria. The HPC provides an estimate of viable heterotrophic bacteria and serves as an indicator of general microbiological water quality (Bartram et al., 2003; Arifan et al., 2018). Total coliforms are commonly used as indicators of potential fecal contamination (Shen et al., 2019; WHO, 2022), with their presence detected by characteristic colonies exhibiting a metallic sheen on m-Endo medium (APHA et al., 2017). The absence of such colonies, together with the HPC result, provides preliminary information on pool water microbiological quality. Routine monitoring of these parameters is therefore important for assessing potential health risks and maintaining a suitable aquatic environment for captive marine mammals (AMMPA, 2026).

Potential Factors Associated with Cutaneous Infection and Implications for Dolphin Health Management

The genus *Staphylococcus* contains several opportunistic bacteria that have been associated with infections in marine mammals. Previous studies have reported the isolation of *Staphylococcus aureus* from bottlenose dolphins maintained under human care (Faires et al., 2009; Mazzariol et al., 2018). Cutaneous infections may be influenced by various factors, including disruption of the skin barrier, host health and immune status, and environmental exposure to microorganisms. However, the specific factors and source of infection in the present case could not be determined, despite the presumptive isolation of *Staphylococcus* sp. from the lesions.

The results should also be considered in the context of antimicrobial resistance (AMR), as aquatic environments may serve as reservoirs for AMR bacteria and antimicrobial resistance genes (ARGs), partly due to anthropogenic inputs such as wastewater discharge, agricultural runoff, and aquaculture activities (Zheng et al., 2021; Siri et al., 2023; Mourão et al., 2025). This highlights the importance of responsible antimicrobial use, water quality management, routine microbiological monitoring, and biosecurity in captive marine mammal facilities. However, AMR genes, resistance mechanisms, and antimicrobial residues were not investigated in this study. In addition, the antimicrobial activity assay was conducted under non-standardized conditions, including the absence of inoculum standardization and the use of NA instead of the recommended medium for conventional susceptibility testing (CLSI, 2024). Therefore, the observed inhibition zones represent preliminary evidence of in vitro antimicrobial activity and cannot be interpreted using standardized susceptible, intermediate, or resistant categories.

From a practical perspective, this case highlights the importance of integrating routine clinical examinations with microbiological monitoring to support early detection and timely veterinary intervention in captive marine mammals (AMMPA, 2026). Regular skin assessments, accompanied by photographic documentation of any abnormal lesions, may facilitate the evaluation of lesion progression and clinical responses to treatment.

When suspicious lesions are identified, bacteriological examination can provide additional information to support veterinary assessment and antimicrobial selection. Furthermore, routine monitoring of pool water quality, appropriate water treatment, sanitation, and biosecurity measures are essential components of preventive health management (Field, 2024; AMMPA, 2026). This current case illustrates how clinical assessment, bacteriological investigation, and environmental monitoring can be used together to support health management decisions in marine mammal facilities.

Study limitations

This case study was limited by the laboratory resources available at Samudra Ancol, particularly for bacteriological and antimicrobial assay methods. Despite these limitations, the results provide preliminary information to support veterinary management and health monitoring in captive marine mammals.

CONCLUSION

Bacteriological examination of cutaneous nodules in an Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) presumptively identified *Staphylococcus* sp. Three months of rifampicin treatment with supportive supplementation resulted in clinical improvement. Pool water examination showed an HPC of 46 CFU/mL, below the facility threshold, with no detectable coliforms. Further standardized antimicrobial assay and molecular identification are recommended.

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AUTHORS CONTRIBUTION

A.P. contributed to data collection, microbiological investigations, administration of antibiotics and supplements, manuscript preparation. L.R. served as the field supervisor, contributed to research supervision, sample examination. A.Y. contributed to materials preparation, sample collection, data collection. A.F.H. contributed to data analysis, manuscript drafting. C.A.A. served as the principal supervisor, contributing to study design, funding acquisition, research supervision, data interpretation, manuscript review and editing. All authors reviewed and approved the final manuscript.

"The authors declare that there is no conflict of interest with the parties involved in this research".

REFERENCES

Alliance of Marine Mammal Parks & Aquariums (AMMPA). 2026. Standards and guidelines for the accreditation of marine mammal facilities. AMMPA. Washington, D. C. p6, p15

American Public Health Association, American Water Works Association, & Water Environment Federation. 2017. Standard methods for the examination of water and wastewater (23rd ed.). APHA Press. Washington, D. C. p9

American Society for Microbiology. 2016. Triple sugar iron agar protocols. https://asm.org/ASM/media/Protocol-Images/Triple-Sugar-Iron-Agar-Protocols.pdf

American Society for Microbiology. 2024. Mannitol salt agar plates protocols. https://asm.org/ASM/media/Protocol-Images/Mannitol-Salt-Agar-Plates-Protocols.pdf

Arifan F, Winarni S, Wahyuningsih, Pudjihastuti I, Broto RTDW. (2018). Total Plate Count (TPC) Analysis of Processed Ginger on Tlogowungu Tourism Village. *Advances in Engineering Research*, 167, 377-379. https://doi.org/10.2991/icoma-18.2019.80

Bartram, J., Cotruvo, J., Exner, M., Fricker, C., & Glasmacher, A. (Eds.). 2003. Heterotrophic plate counts and drinking-water safety: The significance of HPCs for water quality and human health. IWA Publishing. London. p3

Boyd, S. E., Wootton, M., Howe, R. A., Livermore, D. M., MacGowan, A. P., & Kahlmeter, G. (2025). Rifampicin as monotherapy for infections caused by Staphylococcus aureus: Considerations for not applying 'breakpoints in brackets'. *Journal of Antimicrobial Chemotherapy*, 80(12), 3191–3193. https://doi.org/10.1093/jac/dkaf392

Clinical and Laboratory Standards Institute. 2024. *Performance standards for antimicrobial susceptibility testing* (34th ed., CLSI supplement M100). Clinical and Laboratory Standards Institute.

Egwuatu, T. O., Ogunsola, F. T., Okodugha, I. M., Jide, B., Arewa, D. G., & Osinupebi, O. A. (2014). Effect of blood agar from different animal blood on growth rates and morphology of common pathogenic bacteria. *Advances in Microbiology*, 4, 1237-1241. https://doi.org/10.4236/aim.2014.416133

Ewing, R. Y., Sutton, M. N., Herring, H. M., Schubert, M. R., Boyd, D. M., Richardson, J. L., & Rotstein, D. S. (2023). Standardizing gross descriptions of skin lesions in common bottlenose dolphins (*Tursiops truncatus*) stranded in Southwest Florida, 2015–2019. *Frontiers in Marine Science*, 10, 1269075. https://doi.org/10.3389/fmars.2023.1269075

Faires, M. C., Gehring, E., Mergl, J., & Weese, J. S. (2009). Methicillin-resistant *Staphylococcus aureus* in marine mammals. *Emerging Infectious Diseases*, 15(12), 2071-2072. https://doi.org/10.3201/eid1512.090220

Field, C. L. 2024. Management of marine mammals. Merck Veterinary Manual. Rahway.

Gaydos, J. K., St. Leger, J., Raverty, S., Nollens, H., Haulena, M., Ward, E. J., Emmons, C. K., Hanson, M. B., Balcomb, K., Ellifrit, D., Weiss, M. N., & Giles, D. A. (2023). Epidemiology of skin changes in endangered Southern Resident killer whales (*Orcinus orca*). *PLOS ONE*, 18(6), e0286551. https://doi.org/10.1371/journal.pone.0286551

Haughey, R., Hunt, T. N., Hanf, D., Passadore, C., Baring, R., & Parra, G. J. (2021). Distribution and habitat preferences of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) inhabiting coastal waters with mixed levels of protection. *Frontiers in Marine Science*, 8, 617518, 1-20. https://doi.org/10.3389/fmars.2021.617518

Kumari, H., Chakraborti, T., Singh, M., Chakrawarti, M. K., et al. (2020). Prevalence and antibiogram of coagulase negative Staphylococci in bioaerosols from different indoors of a university in India. *BMC Microbiology*, 20, 211. https://doi.org/10.1186/s12866-020-01875-8

Lee, C.-Y., Huang, C.-H., Lu, P.-L., Ko, W.-C., Chen, Y.-H., & Hsueh, P.-R. (2017). Role of rifampin for the treatment of bacterial infections other than mycobacteriosis. *Journal of Infection*, 75(5), 395-408. https://doi.org/10.1016/j.jinf.2017.08.013

Mazzariol, S., Corrò, M., Tonon, E., Biancani, B., Centelleghe, C., & Gili, C. (2018). Death associated to methicillin resistant *Staphylococcus aureus* ST8 infection in two dolphins maintained under human care, Italy. *Frontiers in Immunology*, 9, 2726. https://doi.org/10.3389/fimmu.2018.02726

Mourão, A. V., Fernandes, D., de Sousa, T., Calouro, R., Saraiva, S., Igrejas, G., & Poeta, P. (2025). Aquatic resistome in freshwater and marine environments: Interactions between commensal and pathogenic in the context of aquaculture and One Health. *Microorganisms*, 13(7), 1591, 1-11. https://doi.org/10.3390/microorganisms13071591

Romani-Cremaschi, U., Rebollada-Merino, A., Canales, R., Vargas-Castro, I., Pérez-Sancho, M., Sánchez-Vizcaíno, J. M., Domínguez, M., Domínguez, L., & Rodríguez-Bertos, A. (2023). Histopathology, immunohistochemical diagnosis, and management of penicillin-resistant *Staphylococcus delphini* cutaneous infection in a bottlenose dolphin. *Aquatic Mammals*, 49(2), 117-120. https://doi.org/10.1578/AM.49.2.2023.117

Sánchez Martínez, L., Fernández Ruenes, G., Guevara March, C., Cruz Martínez, D., Sánchez Campos, L., López León, N., López Cañizares, R., & Campos Talavera, R. (2015). Similar cutaneous lesions observed in three dolphins *Tursiops truncatus* from the National Aquarium of Cuba: Case studies. *REDVET. Revista Electrónica de Veterinaria*, 16(10), 1–12.

Schleifer, K.-H., & Bell, J. A. 2015. *Staphylococcus*. In Bergey's Manual of Systematics of Archaea and Bacteria. *Wiley*, p1. https://doi.org/10.1002/9781118960608.gbm00569

Segura-Göthlin, S., Fernández, A., Arbelo, M., Andrada Borzollino, M. A., Felipe-Jiménez, I., Colom-Rivero, A., Fiorito, C., & Sierra, E. (2023). Viral skin diseases in odontocete cetaceans: Gross, histopathological, and molecular characterization of selected pathogens. *Frontiers in Veterinary Science*, 10, 1188105. https://doi.org/10.3389/fvets.2023.1188105

Shen Q, Wang X, Chen E, Ma Z. 2019. Comparative Study on Test Methods of Total Coliforms in Domestic Drinking Water. *IOP Conf. Series: Earth and Environmental Science*, 310, 042002. https://doi.org/10.1088/1755-1315/310/4/042002

Siri, Y., Precha, N., Sirikanchana, K., Haramoto, E., & Makkaew, P. (2023). Antimicrobial resistance in southeast Asian water environments: A systematic review of current evidence and future research directions. *Science of the Total Environment*, 896, 165229, 1-15. https://doi.org/10.1016/j.scitotenv.2023.165229

Thakur, P., Nayyar, C., Tak, V., & Saigal, K. (2017). Mannitol-fermenting and tube coagulase-negative staphylococcal isolates: Unraveling the diagnostic dilemma. *Journal of Laboratory Physicians*, 9(1), 65-66. https://doi.org/10.4103/0974-2727.187926

Tille, P. M. 2017. Bailey & Scott's diagnostic microbiology (14th ed.). Elsevier. Amsterdam. p30-60. https://www.elsevier.com/books/bailey-and-scotts-diagnostic-microbiology/tille/978-0-323-35482-0

Varaldo, P. E., Kilpper-Bälz, R., Biavasco, F., Satta, G., & Schleifer, K. H. (1988). *Staphylococcus delphini* sp. nov., a coagulase-positive species isolated from dolphins. *International Journal of Systematic Bacteriology*, 38(4), 436-439. https://doi.org/10.1099/00207713-38-4-436

WorldA Health Organization. 2022. Guidelines for drinking-water quality: Fourth edition incorporating the first and second addenda. World Health Organization. Jenewa. p315.

Yeh, E., Pinsky, B. A., Banaei, N., & Baron, E. J. (2009). Hair sheep blood, citrated or defibrinated, fulfills all requirements of blood

agar for diagnostic microbiology laboratory tests. *PLOS ONE*, 4(7), e6141. https://doi.org/10.1371/journal.pone.0006141

Zheng, D., Yin, G., Liu, M., Chen, C., Jiang, Y., Hou, L., & Zheng, Y. (2021). A systematic review of antibiotics and antibiotic resistance genes in estuarine and coastal environments. *Science of the Total Environment*, 777, 146009. https://doi.org/10.1016/j.scitotenv.2021.146009