

Identification of *Vibrio* Species and Predominant Cholera-Causing Serotypes at Hospital Semporna

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Abstract

Semporna, Sabah has reported many cholera outbreaks in recent years, posing a significant threat to the public's health. Despite that, the current knowledge regarding cholera in Semporna is limited, as there is not enough data on the incidence and prevalence of the disease. This study aimed to identify the *Vibrio* species isolated from suspected cholera cases, determine the predominant *Vibrio cholerae* serotypes, and describe the demographic characteristics regarding age, gender, and nationality of confirmed cholera cases at Hospital Semporna in 2023. A retrospective descriptive observational study was conducted using laboratory records of patients with suspected cholera between January and December 2023. Data were gathered, focusing on patient demographics and cholera positivity. A total of 286 suspected cholera cases were reviewed, of which 33 were confirmed as *V. cholerae* O1, with no fatalities reported. More than half (63.6%, 21/33) of the confirmed cases occurred in children aged below 10 years. Males accounted for 54.5% (18/33) of the cases, while non-Malaysians represented 63.6% (21/33). All 33 cases were caused by the *V. cholerae* O1 Ogawa, whereas the Inaba, Hikojima, and O139 serotypes were not detected. In conclusion, *V. cholerae* O1 Ogawa was the predominant serotype responsible for cholera cases in Hospital Semporna during the study period. Given its susceptibility to cholera outbreaks, the results make it even clearer that ongoing surveillance, targeted public health interventions, and preventive measures are needed to lower the risk of future outbreaks and keep the local population safe.

Keywords: Cholera outbreak; patient demographics; Semporna; serotypes; *Vibrio cholerae*

INTRODUCTION

Cholera is a severe gastrointestinal disease caused by *Vibrio cholerae*, a bacterial pathogen with over 200 serogroups, of which O1 and O139 are linked to epidemic and pandemic cholera in humans. The O1 serogroup is further classified into the Ogawa, Inaba, and the rare Hikojima

serotypes, of which Ogawa and Inaba are the most frequently isolated in clinical settings. In Malaysia, cholera is considered non-endemic in most states, with more than 90% of reported cases occurring in Sabah (Lopez *et al.*, 2020). Previous outbreaks in Malaysia have consistently identified *V. cholerae* O1, particularly the Ogawa serotype, as the predominant strain responsible for cholera cases. Previous outbreaks in Kelantan, Terengganu, Sarawak, and Sabah have consistently identified *V. cholerae* O1, particularly the Ogawa serotype, as the predominant strain responsible for cholera cases (Ang *et al.*, 2010; Bilung *et al.*, 2014; Teh *et al.*, 2012; Zaw *et al.*, 2019). These findings highlight the continued predominance of the O1 Ogawa serotype in Malaysia and highlight the importance of continuous surveillance to monitor circulating serotypes and support effective cholera prevention and control strategies.

Despite improvements in public health, cholera remains a significant concern in coastal districts of Sabah, particularly Semporna, where environmental and socio-economic factors such as inadequate sanitation, a tropical climate, and reliance on fishing contribute to the persistence of waterborne diseases. Although several studies have investigated cholera outbreaks in Sabah, they have mainly focused on districts such as Kudat, Beluran, and Sandakan (Muhammad *et al.*, 2019; Zaw *et al.*, 2019), leaving limited epidemiological information for Semporna. Therefore, this study aims to assess *V. cholerae* infections and determine the predominant serotypes among patients with suspected cholera at Hospital Semporna. In addition, the demographic characteristics of confirmed cholera cases are described to provide a better understanding of the local epidemiology and will help mitigate the impact of future outbreaks and strengthen public health efforts in Semporna and beyond.

METHODOLOGY

Study Design

This retrospective descriptive observational study was conducted using laboratory records of patients with suspected cholera at Hospital Semporna, Sabah, between January and December 2023.

Sample Size

The sample size for this study was calculated using the Raosoft calculator, employing a 5% margin of error and a 95% confidence level derived from a population of 286 patients. The suggested sample size was 165. The study included all 286 patients who met the inclusion and exclusion criteria set in this study. Following laboratory analysis, 33 patients were confirmed to have *V. cholerae* infection and were included in the demographic and serotype analyses presented in the Results and Discussion section.

Inclusion and Exclusion Criteria

Patients were included if they (i) presented with clinical symptoms suggestive of cholera, (ii) had a rectal swab requested by the clinician for *V. cholerae* culture, and (iii) had the specimen collected at Hospital Semporna from either the Emergency Department or wards.

Patients were excluded if they (i) presented with acute bloody diarrhea suggestive of an alternative diagnosis, (ii) had received antibiotic treatment before specimen collection as documented on the laboratory request form, or (iii) were environmental samples submitted by the Semporna District Health Office.

Isolation and Identification of *Vibrio cholerae*

Rectal swab specimens were collected from patients with suspected cholera as part of routine clinical care at Hospital Semporna. The process began with selective cultivation on Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar, which promoted the growth of *V. cholerae* and other *Vibrio* species while inhibiting unwanted bacterial contaminants. Suspected colonies were then sub-cultured on non-selective Mueller-Hinton agar (MHA) for further testing, including antibiotic susceptibility analysis. Several biochemical tests were performed to confirm the presence of *V. cholerae*, including the oxidase test, which distinguished *Vibrio* from Enterobacteriaceae. Differential media tests such as the Triple Sugar Iron (TSI) and bile esculin tests helped to differentiate *V. cholerae* from other bacterial species such as *Aeromonas* species. Finally, serotyping was conducted using polyvalent antisera (Ogawa and Inaba) to determine the predominant serotype of *V. cholerae* in the sample.

Data Collection

All data were extracted from the laboratory electronic archive and hospital records. The final 286 patients were concluded following data cleaning, which among others, excluded all patients with exclusion criteria and eliminated duplicate data of the same patient. Demographic information, including age, gender, and nationality, together with laboratory findings and *V. cholerae* serotypes, were extracted for analysis. Data regarding sanitary practices was obtained from the Semporna District Health Office. This covered information on patients' access to clean water and sanitation, critical elements influencing cholera transmission in the area.

Data Analysis

The cleaned data were analyzed using the Statistical Package for the Social Sciences (SPSS) Version 29.0.1.0. The demographics data of age, gender, and nationality were analyzed for their association with cholera infection. The associations between demographic variables and laboratory-confirmed cholera infection were analysed using the Kruskal-Wallis H test for age and the Pearson Chi-square test for gender and nationality. A p-value of < 0.05 was considered statistically significant for all analyses.

Ethics

Ethical approval was granted by the Research Ethics Committee, Faculty of Health Sciences (EC000569). All patient information was anonymized prior to analysis to protect confidentiality.

RESULTS AND DISCUSSION

The findings revealed that young children were the most impacted demographic (Table 1). A similar tendency was observed in India and Bangladesh (Khan *et al.*, 2020; Sharma *et al.*, 2017). Children have lower immune systems than adults, rendering them more vulnerable to cholera. However, age was not significantly associated with laboratory-confirmed cholera infection (Kruskal-Wallis H test, $p = 0.105$). No significant association was observed between gender and laboratory-confirmed cholera infection (Pearson Chi-square test, $p = 0.898$), suggesting that males and females were similarly affected. On the other hand, nationality showed a significant association with laboratory-confirmed cholera infection (Pearson Chi-square test, $p = 0.003$). Non-locals constituted 63.6% of the cholera cases in Semporna. It may be due to the regular cross-border migrations of individuals between Malaysia and the Philippines (Maluda *et al.*, 2024). These findings highlight the significance of confronting disparities associated

with nationality while also emphasizing the necessity for comprehensive public health initiatives that encompass all demographic categories.

Table 1 Demographic data of cholera cases in Hospital Semporna

Demographic Data	Cholera, n (%)
Age	
0-9	21 (63.6)
10-19	5 (15.2)
20-29	1 (3.0)
30-39	2 (6.1)
40-49	0 (0.0)
50-59	1 (3.0)
60-69	2 (6.1)
70-79	1 (3.0)
80-89	0 (0.0)
Gender	
Male	18 (54.5)
Female	15 (45.5)
Nationality	
Local	12 (36.4)
Non-local	21 (63.6)
Death	0 (0.0)

V. cholerae O1 Ogawa strain caused all confirmed cases in Semporna emphasizes its major importance as the main etiological agent of cholera in the area (Table 2). The Inaba, Hikojima, and O139 serotypes were not associated with any cases. The Ogawa serotype has higher toxicity and gene expression compared to the Inaba serotype, which may explain why it is more common in nature.

Table 2 Serotype frequencies and percentages

Serotype	Frequency, n (%)
O1	
Ogawa	33 (100)
Inaba	0 (0)
Hikojima	0 (0)
O139	0 (0)

Cholera transmission started in June, reached its peak in August, and decreased by September and October (Figure 1). This pattern corresponds with the dry season in Semporna (April to October), in contrast to the monsoon season from November to March. This means that cholera outbreaks in this area might not be directly linked to the monsoon rains. Instead, other environmental or behavioral variables during the dry season may exert a more substantial influence. Elevated temperatures (26 °C to 28.9 °C) and salt levels of around 15% significantly contribute to cholera epidemics by promoting the adhesion of *V. cholerae* to small aquatic organisms such as copepods (Noor *et al.*, 2015). These factors emphasize the increased likelihood of cholera outbreaks during the dry season, accentuating the necessity for initiatives to enhance water accessibility, sanitation, and hygiene practices in the area.

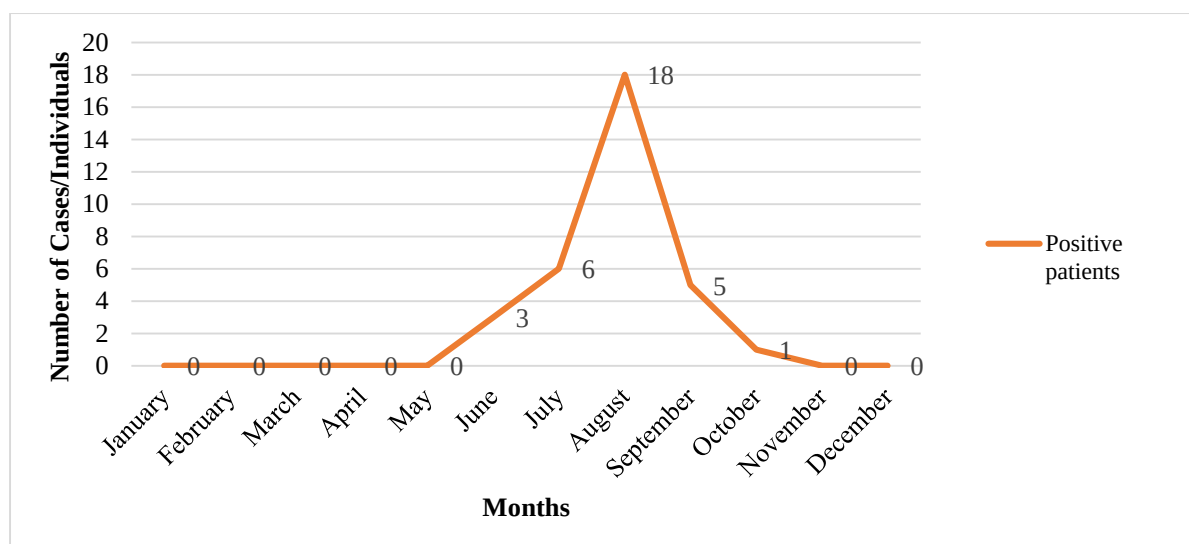


Figure 1 Trend of cholera cases in Hospital Semporna by month in 2023

CONCLUSION

This study successfully assessed *V. cholerae* infections and determined Ogawa as the prevalent serotype among patients at Hospital Semporna in 2023. Although age and gender did not show a significant correlation with cholera infections, nationality proved to be a crucial determinant. The findings highlight significant public health consequences, necessitating targeted interventions such as immunization with Oral Cholera Vaccines (OCV) for high-risk populations, enhanced access to potable water, improved sanitation, and educational initiatives on hygiene habits. These techniques are crucial for reducing cholera risks and averting future outbreaks in at-risk areas.

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REFERENCES

- Ang, G. Y., Yu, C. Y., Balqis, K., Elina, H. T., Azura, H., Hani, M. H., & Yean, C. Y. (2010). Molecular evidence of cholera outbreak caused by a toxigenic *Vibrio cholerae* O1 El tor variant strain in Kelantan, Malaysia. *Journal of Clinical Microbiology*, 48(11), 3963–3969. <https://doi.org/10.1128/jcm.01086-10>
- Bilung, L. M., Fuh, Y. S., Linang, V., Benjamin, A., Vincent, M., Apun, K., Lihan, S., & Lin, C. S. (2014). Genomic diversity of cholera outbreak strains in East Malaysia. *Malaysian Journal of Medicine and Health Sciences*, 10(2), 19–26. https://medic.upm.edu.my/upload/dokumen/FKUSK1_Jurnal_Text_Article_3.pdf
- Khan, A. I., Rashid, M. M., Islam, M. T., Afrad, M. H., Salimuzzaman, M., Hegde, S. T., & Qadri, F. (2020). Epidemiology of cholera in Bangladesh: Findings from nationwide

- hospital-based surveillance, 2014–2018. *Clinical Infectious Diseases*, 71(7), 1635–1642. <https://doi.org/10.1093/cid/ciz1075>
- Lopez, A. L., Dutta, S., Qadri, F., Sovann, L., Pandey, B. D., Wan Mansor Hamzah, Memon. I, Iamsirithaworn S, Dang DA, Chowdhury F, Heng S, Kanungo S, Mogasale V, Sultan A, Ylade M. (2020). Cholera in selected countries in Asia. *Vaccine*, 38, A18–A24. <https://doi.org/10.1016/j.vaccine.2019.07.035>
- Maluda, M. C. M., Johnson, E., Robinson, F., Muhammad Jikal, Fong, S. Y., Mohammad Jeffree Saffree, Fornace, K.M., & Kamruddin Ahmed (2024). The incidence, and spatial trends of cholera in Sabah over 15 years: Repeated outbreaks in coastal areas. *PLOS Global Public Health*, 4(1). <https://doi.org/10.1371/journal.pgph.0002861>
- Muhammad Jikal, Tuan Riduan, Rosmawati Aarifin, Mohammad Saffree Jeffree & Kmrudin Ahmed (2019). Cholera outbreak by sea gypsies in Sabah, Malaysia: A challenge in North Borneo. *International Journal of Infectious Diseases*, 83, 83–85. <https://doi.org/10.1016/j.ijid.2019.04.008>
- Noor Artika Hassan. Jamal Hisham Hashim, Capon, A., & Mohd Shahrul Faisal (2015). The impacts of climate change on cholera cases in Malaysia: A case study of Sabah. *In ISEE Conference Abstracts 27*, 2015(1), 1386
- Sharma, A., Dutta, B. S., Rasul, E. S., Barkataki, D., Saikia, A., & Hazarika, N. K. (2017). Prevalence of *Vibrio cholerae* O1 serogroup in Assam, India: A hospital-based study. *Indian Journal of Medical Research*, 146(3), 401–408. https://doi.org/10.4103/ijmr.IJMR_631_15
- Teh, C. S. J., Zarizal Suhbaili, Lim, K. T., Muhamad Afif Khamaruddin, Fariha Yahya, Mohd Hailmi Sajili, Yeo, C.C. & Thong, K. L. (2012). Outbreak-associated *Vibrio cholerae* genotypes with identical pulsotypes, Malaysia, 2009. *Emerging Infectious Diseases*, 18(7), 1177–1179. <https://doi.org/10.3201/eid1807.111656>
- Zaw, M. T., Nor Amalina Enran, Mohd Yusof Ibrahim, Maria Suleiman, Tajul Ariffin Awang Mohd, Aza Sherin Yusuff, Naing, K.S., Myint, T., Muhammad Jikal, Mohd Azmi Salleh & Lin, Z. (2019). Genetic diversity of toxigenic *Vibrio cholerae* O1 from Sabah, Malaysia 2015. *Journal of Microbiology, Immunology and Infection*, 52(4), 563–570. <https://doi.org/10.1016/j.jmii.2018.01.003>