Jurnal Kesehatan Komunitas Indonesia (JKKI)
Volume 6 Issue 2, August 2026, pp 448-460
https://ebsina.or.id/journals/index.php/jkki
eISSN 2503-2801, pISSN 2985-3435

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Kingella denitrificans as a Mimicker of *Neisseria meningitidis* in Carriage Surveillance

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Article History
Submitted: 02-07-2026
Revised: 17-07-2026
Accepted: 27-07-2026

doi.org/10.58545/jkki.v6i2.871

Abstract

Background: Meningitis caused by *Neisseria meningitidis* remains a significant public health concern. A carriage study is crucial for understanding the distribution of *Neisseria meningitidis* serogroups in the community. The presence of *Kingella denitrificans* may lead to misidentification as *N. meningitidis* due to phenotypic similarity on blood agar. However, the prevalence of *K. denitrificans* in nasopharynx and oropharynx remains unclear. **Objective:** This study aimed to determine the prevalence of *K. denitrificans* as a mimicker of *N. meningitidis* in a carriage surveillance setting and to evaluate the role of MALDI-TOF MS in ensuring accurate bacterial identification. **Methods:** A cross-sectional study was conducted from September to December 2025 among unvaccinated adults in Jakarta with meningococcal disease. Oropharyngeal and nasopharyngeal swabs were collected and cultured on VCNT-supplemented blood agar. Preliminary identification was performed using colony morphology, Gram staining, and oxidase and catalase tests. Definitive identification was carried out using MALDI-TOF MS. **Results:** Among 228 participants, 11 (4.8%) carried *Kingella spp.* in the nasopharynx and/or oropharynx. All carriers were females aged 19–33 years (median age: 25 years). The isolates exhibited colony morphology similar to *N. meningitidis*, potentially leading to misidentification. However, they could be differentiated by their Gram staining morphology and catalase test results. MALDI-TOF MS identified 5 of 11 isolates (45.5%) as *K. denitrificans* with high-confidence log scores. **Conclusion:** *K. denitrificans* phenotypically might mimicked *N. meningitidis* in carriage surveillance but can be distinguished with Gram staining. The integration of MALDI-TOF MS is essential to improve diagnostic accuracy and prevent false-positive results. Further studies are needed to assess the feasibility of implementing this technology in resource-limited settings.

Keywords

Kingella denitrificans, Oropharyngeal swabs, Nasopharyngeal swabs, Carriage, MALDI-TOF MS

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How to cite:
Lestari, D. C., Kemalasari, P. A., Kamila, R., Ejoli, C., Tafroji, W., Ariyanto, I. A., Shahab, S. N., Shelby, B., & Arumsari, A. (2026). *Kingella denitrificans* as a Mimicker of *Neisseria meningitidis* in Carriage Surveillance in Jakarta, Indonesia. Jurnal Kesehatan Komunitas Indonesia, 6(2), 448-460. https://doi.org/10.58545/jkki.v6i2.871

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1. BACKGROUND

Meningitis, a life-threatening inflammation of the meninges surrounding the central nervous system, represents a critical global public health challenge (World Health Organization [WHO], 2015). *Neisseria meningitidis* remains the predominant etiological agent of bacterial meningitis, contributing substantially to morbidity and mortality worldwide (Venkatesan, 2021). The burden is particularly pronounced in low- and middle-income countries, where mortality rates can exceed 50% despite available interventions (Hasbun, 2022). In the Asia-Pacific region, although the overall incidence of invasive meningococcal disease remains relatively low (‹0.2 cases per 100,000 population), surveillance data indicate persistent transmission and disease occurrence (Aye et al., 2020).

Transmission of *N. meningitidis* occurs through respiratory droplets and direct contact with infected secretions (Centers for Disease Control and Prevention [CDC], 2026). Importantly, nasopharyngeal colonization typically manifests as asymptomatic carriage rather than invasive disease, establishing the upper respiratory tract as the primary reservoir for bacterial transmission (Balmer et al., 2018). Carriage prevalence varies considerably across populations and settings, with systematic reviews reporting rates ranging from 1.4% to 14.2% in Asian countries and higher rates observed among adolescents and young adults (Serra et al., 2020). Understanding carriage dynamics is essential for informing vaccination strategies and predicting disease outbreaks, as carriage studies provide critical insights into the distribution of meningococcal serogroups within communities (Peterson et al., 2018).

Standard surveillance protocols for detecting *N. meningitidis* carriage rely on culture-based methods using selective media supplemented with VCNT (vancomycin, colistin, nystatin, and trimethoprim) to enhance isolation specificity (Miellet et al., 2021; Ningsih & Sumadiyo, 2025). Both oropharyngeal and nasopharyngeal specimens are recommended for optimal detection sensitivity (Roberts et al., 2009). However, a significant diagnostic challenge arises from the phenotypic similarity between *N. meningitidis* and other commensal organisms, particularly *Kingella denitrificans*, which can also proliferate on VCNT-supplemented media (Auwaerter, 2022).

Kingella denitrificans, a Gram-negative coccobacillus belonging to the family *Neisseriaceae*, shares several microbiological characteristics with *N. meningitidis*, including oxidase positivity and similar

colony morphology on blood agar (Auwaerter, 2022; The Royal College of Pathologists, 2025). This phenotypic convergence poses a substantial risk of misidentification during routine microbiological investigations, potentially leading to false-positive results for meningococcal carriage. Such misidentification carries serious implications for clinical management and public health response, including unnecessary outbreak investigations, inappropriate antibiotic prophylaxis for contacts, and inaccurate estimation of meningococcal carriage burden (The Royal College of Pathologists, 2025).

Differentiation of *K. denitrificans* from *N. meningitidis* traditionally requires additional biochemical testing, including a catalase reaction and assessment of Gram stain morphology, as *Kingella* species typically exhibit bacillary rather than diplococcal forms (Auwaerter, 2022). However, these conventional methods are time-consuming and may lack sufficient discriminatory power, particularly in resource-limited settings. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a rapid, accurate, and cost-effective alternative for bacterial identification, offering superior performance compared to conventional phenotypic methods (Rocca et al., 2025).

Despite recognition of the misidentification risk, the prevalence of *K. denitrificans* in meningococcal carriage surveillance remains poorly characterized, particularly in Southeast Asian populations. This study aimed to determine the prevalence of *K. denitrificans* among unvaccinated adults in Jakarta, Indonesia, and to evaluate the utility of MALDI-TOF MS in distinguishing *Kingella* species from *N. meningitidis* in carriage surveillance settings.

2. METHODS

A cross-sectional descriptive study was conducted from September to December 2025 to determine the prevalence of *K. denitrificans* among adults in Jakarta with no prior history of meningococcal vaccination. A total of 228 participants were recruited from the study population using a convenience sampling approach, met the inclusion criteria, and provided informed consent. This approach allows the assessment of bacterial carriage at specific points in time without clinical intervention. Oropharyngeal and nasopharyngeal swabs were collected using FLOQSwabs® (Copan Diagnostics, Murrieta, CA), inoculated into skim milk–tryptone–glucose–glycerol (STGG) as

transport media, and maintained at a temperature of ≤4°C during specimen transport. The specimen was then stored at -80°C before processing. 100 µl of the specimens were inoculated onto blood agar supplemented with Vancomycin, Colistin, Nystatin, and Trimethoprim (VCNT) and incubated at 37°C with 5% CO2 for 24–48 hours.

Initial screening of isolates was performed based on colony morphology and Gram staining. Definitive identification of suspect isolates was performed using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) (Bruker Daltonics, Bremen, Germany). Briefly, a single colony from a fresh culture was applied directly to a polished steel target plate, coated with 1 µL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution, and air-dried. Mass spectra were acquired and evaluated against a reference database. Identification confidence was determined based on the manufacturer's log score threshold. The MALDI-TOF score ≥ 2.00 is reliable for species level, while 1.7 to 1.99 is reliable for genus level.

This project was approved by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (Ethical number. KET-629/ UN.2F1/ ETIK/ PPM. 00.02/2025). Before specimen collection, written informed consent was obtained from all participants. All data were anonymized to ensure participant confidentiality.

3. RESULTS

Out of 228 subjects, 11 subjects (4.8%) carried *Kingella sp.* in their nasopharyngeal and/or oropharyngeal flora. Based on the demographic analysis of the study subjects, all those who tested positive for *Kingella sp.* were female, aged 19–33 years (median, 25 years old) (**Table 1**). These isolates initially posed a diagnostic challenge due to their phenotypic similarity to *N. meningitidis*. On blood agar, *Kingella sp.* appeared as small, grayish-white, smooth colonies, which are similar to the typical morphology of commensal *Neisseria sp.*, including *N. meningitidis* (**Figure 1**).

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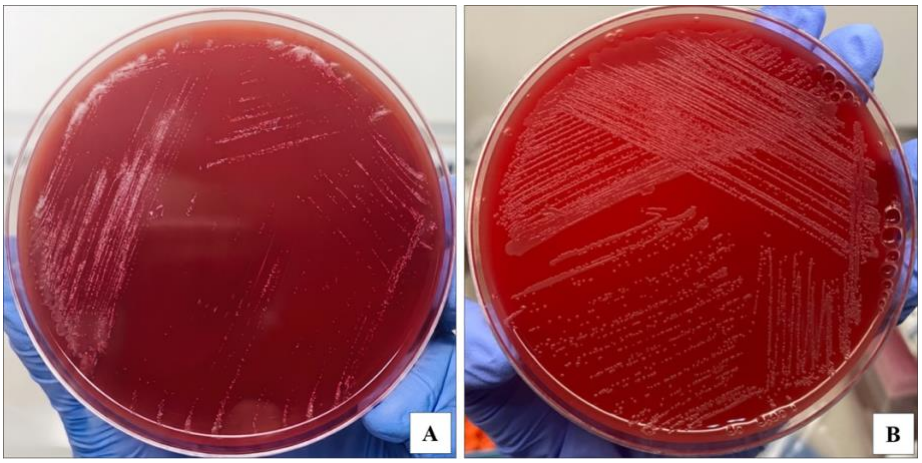

Figure 1. Bacterial growth on blood agar supplemented with VCNT. (A) *K. denitrificans*, and (B) *N. meningitidis*

Gram staining showed that *K. denitrificans* consisted of Gram-negative bacilli, often occurring in pairs, while *N. meningitidis* exhibited the typical Gram-negative, coffee-bean-shaped diplococcal morphology (**Figure 2**).

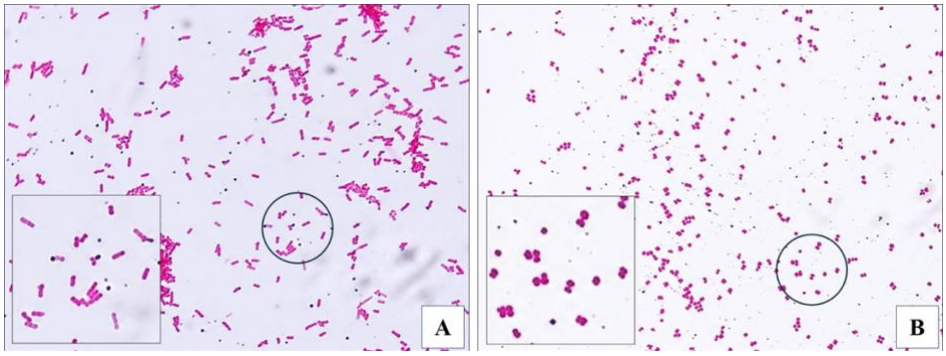

Figure 2. Gram staining of (A) *K. denitrificans*; (B) *N. meningitidis*

Oxidase test results are presented in **Figure 3**, and catalase test results are displayed in **Figure 4**.

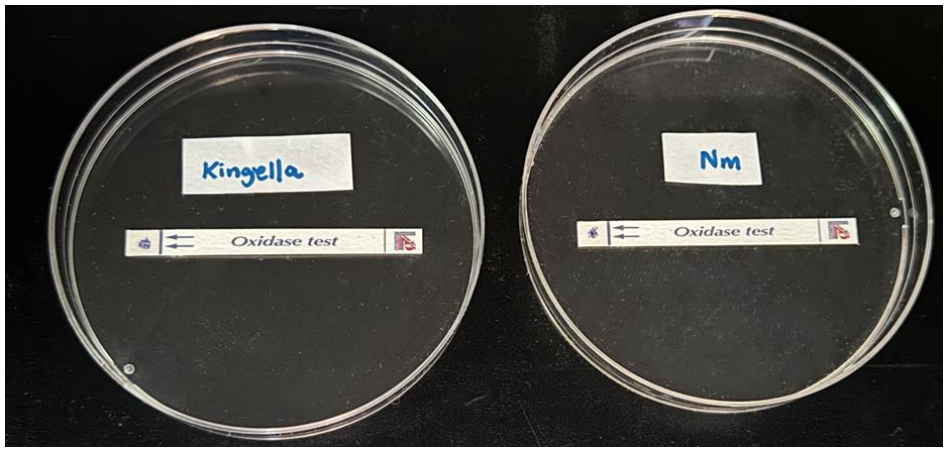

Figure 3. Positive oxidase test results of (A) *K. denitrificans*; (B) *N. meningitidis*

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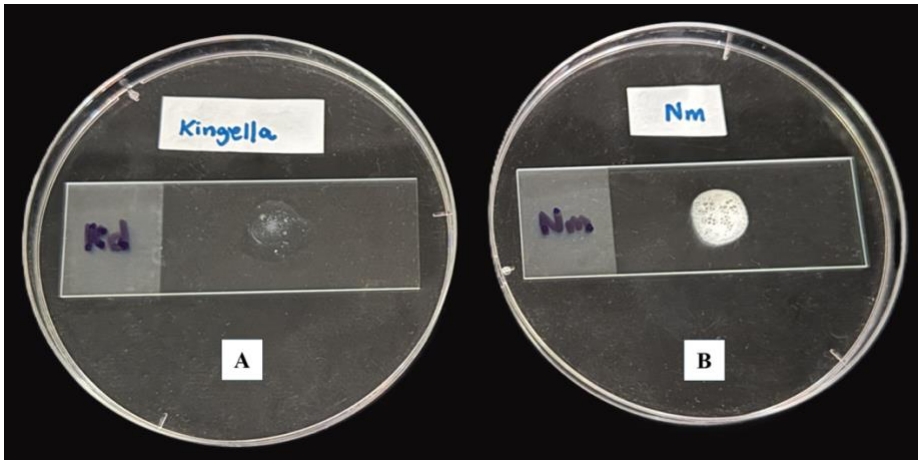

Figure 4. Negative catalase test result of (A) *K. denitrificans*; Positive catalase test result of (B) *N. meningitidis*

These preliminary findings suggested that Gram staining can be used to distinguish *Kingella* sp. from *N. meningitidis*. To support this Gram staining results, we confirmed with MALDI-TOF MS resulting in *K. denitrificans* from other members of the Neisseriaceae family with high reliability scores. The definitive identification results, including the high-confidence MALDI-TOF MS scores, are presented in **Table 1**.

Table 1. MALDI-TOF MS Score Values for Identification of K. denitrificans

Subject Code	Age (years)	Value	Identification by MALDI-TOF
A051J	25	1.95	*Kingella sp.*
A067J	25	1.90	*Kingella sp.*
A080J	26	2.00	*Kingella denitrificans*
A091J	25	1.92	*Kingella sp.*
A101J	33	2.25	*Kingella denitrificans*
A155J	30	2.01	*Kingella denitrificans*
A166J	20	1.91	*Kingella sp.*
A211J	21	1.85	*Kingella sp.*
A212J	21	1.96	*Kingella sp.*
A219J	19	2.01	*Kingella denitrificans*
A220J	21	2.21	*Kingella denitrificans*

4. DISCUSSION

This study highlights the presence of *Kingella sp.* and the possible misidentification of *N. meningitidis* based on colony morphology within a surveillance framework in Jakarta. Our findings revealed that the normal commensal flora (i.e., *K. denitrificans*) may interfere with *N. meningitidis* screening by yielding presumptive-positive results that require confirmatory testing. This emphasizes the need for further identification testing rather than colony appearance to distinguish *Kingella sp.* and *N. meningitidis*. Both exhibit similar colony appearance and color, but can be distinguished by Gram

staining. However, confirmation of the bacterial species requires additional identification procedures, as species-level identification cannot be established solely based on Gram stain results. Vancomycin, Colistin, Nystatin, and Trimethoprim, as selective agents, failed to suppress the growth of *Kingella sp.* on VCNT blood agar media. This might lead to misidentification in clinical microbiology laboratories. These results underscore that relying solely on conventional culture methods is insufficient for accurate surveillance, highlighting the need for more robust identification techniques, such as MALDI-TOF MS or genotypic assays, to prevent false-positive reporting.

Taxonomically, *K. denitrificans* and *N. meningitidis* belong to the same family, *Neisseriaceae*, but are classified into separate genera, *Kingella* and *Neisseria*, respectively. However, recent phylogenetic studies reveal that the classification within the genus *Kingella* remains highly dynamic, exhibiting both paraphyly and polyphyly (Morreale et al., 2023). This suggests close genetic proximity and even overlap among members of the *Neisseriaceae* family. Within a more specific scope, the genus *Kingella* consists of five primary taxa: *K. potus*, *K. oralis*, *K. denitrificans*, *K. negevensis*, and *K. kingae* (Dewhirst et al., 1989; Giacomini et al., 2025). These species form a major clade within the *Neisseriaceae* family, phylogenetically most closely related to the genus *Neisseria*. As with other members of the *Neisseriaceae*, all *Kingella* species are common inhabitants of the human oral and oropharyngeal microbiota (Giacomini et al., 2025).

Conventionally, *N. meningitidis* and *K. denitrificans* are isolated on chocolate agar. These two species exhibit very similar phenotypic characteristics when grown on the same plate, including smooth, round, moist, and uniform colony morphology and grayish pigmentation. In addition to their morphological similarities, these two pathogens share the same habitat in the nasopharynx.

K. denitrificans is a commensal flora of the oropharynx or nasopharynx that is more commonly found in healthy humans. In the complex ecosystem of the human respiratory tract, commensal bacteria can become dominant bacteria and compete with other microorganisms for nutrients (McElvania TeKippe, 2016). *N. meningitidis* is a Gram-negative aerobic bacterium and a primary causative agent of bacterial meningitis and invasive sepsis, contributing to significant morbidity and mortality worldwide. This bacterium naturally colonizes the human upper respiratory tract, particularly the oropharyngeal region, and is transmitted

between individuals via airborne droplets. The majority of these colonizations remain asymptomatic, with carriers serving as the primary reservoir for transmission within the population (Read, 2014).

These two bacteria inhabit the same niche and possess similar phenotypic features. However, they display differing results in the catalase biochemical test. This variation in catalase reactivity reflects how each bacterium copes with oxidative stress in the respiratory tract environment (Valenza et al., 2007). The invasive pathogen *N. meningitidis* secretes the catalase enzyme as a virulence factor to neutralize free radicals generated by the host immune system to support its survival. On the other hand, *K. denitrificans* functions as a commensal flora and relies on a more efficient non-catalase defense mechanism for survival and nutrient acquisition. Therefore, physiological analysis justifies the positive catalase result for *N. meningitidis* and the negative result for *K. denitrificans* (Valenza et al., 2007). This catalase test could also distinguish *Kingella sp.* from *N. meningitidis*.

To overcome the phenotypic ambiguities between these species, MALDI-TOF MS serves as a robust diagnostic tool for definitive identification (Singhal et al., 2015). The process involves ionizing a sample aliquot within a chemical matrix using short laser pulses, and the resulting ions are analyzed by their mass-to-charge (m/z) ratio (Li et al., 2022). The generated protein spectrum, primarily composed of highly conserved ribosomal proteins, is then compared against an extensive spectrometer database to provide precise identification (Li et al., 2022).

In this study, the analyzed isolates yielded high-confidence MALDI-TOF MS log scores ranging from 1.85 to 2.25 for *Kingella sp.* (Table 1). 5 of 11 isolates (45.5%) had scores› 2.00, definitively confirming the identity of *K. denitrificans* by precisely matching their unique proteomic fingerprint against the reference database. By achieving values well above the standard species-level identification threshold, this proteomic validation effectively bypassed the limitations and ambiguities of subjective biochemical interpretations, ensuring robust differentiation from *N. meningitidis* across recovered samples (Calderaro & Chezzi, 2024). Six out of 11 isolates (54.5%) had scores between 1.85 and 1.96, which are considered reliable at the genus level. The main advantage of MALDI-TOF MS in identifying these two bacteria lies in its time efficiency and high accuracy in differentiating *Kingella sp.*, including *K. denitrificans*, from *N. meningitidis* directly

from pure colonies. This tool is equipped with a 96-well target plate, enabling the analysis of large numbers of samples simultaneously (high throughput) in a single work cycle. Therefore, MALDI-TOF MS is a crucial instrument for definitive bacterial identification. This ability also overcomes the major limitations of conventional phenotypic tests, which are often subjective, require long incubation times, and are susceptible to misidentification due to the morphological similarities between the two species (Branda et al., 2014).

5. CONCLUSION

In this meningococcal carriage surveillance, *K. denitrificans* emerged as a prevalent commensal and showed a similar appearance to *N. meningitidis* on VCNT-supplemented blood agar media. Even though Gram staining can distinguish *Kingella sp.* from *N. meningitidis*, integrating MALDI-TOF MS into surveillance workflows is essential to prevent misidentification and false-positive reporting. Future studies should assess the cost-effectiveness and scalability of such confirmatory strategies in resource-limited settings.

AUTHOR CONTRIBUTIONS

DCL: Conceptualization, formal analysis, methodology, investigation, resources, data curation, writing—review and editing, visualization, supervision, and funding acquisition. PAK: Formal analysis, investigation, writing-original draft preparation, visualization, and project administration. RK: Formal analysis, writing-original draft preparation, and visualization. CE: Writing-original draft preparation, and visualization. WT: Methodology, investigation, writing-review and editing, and visualization. IAA and SNS: Investigation, resources, and writing-review and editing. BS and AA: Investigation, data curation, and project administration. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENT

This study was supported by ISAC Grant Project 2024.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data can be accessed from the corresponding author upon reasonable request.

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