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Pseudomonas aeruginosa carriage and associated risk factors in healthy individuals and patients from Rotterdam, Rome, and Jakarta

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***Pseudomonas aeruginosa* may colonize humans, however, epidemiological data are scarce. Here, we determined overall and body site-specific carriage rates and associated risk factors among healthy individuals and newly admitted patients in three major cities. This cross-sectional study was conducted in Rotterdam (The Netherlands), Rome (Italy), and Jakarta (Indonesia) between 2022–2024. Adult healthy individuals and newly admitted patients were asked to provide throat, navel, and rectal/perianal swabs, and to complete a questionnaire. Univariable and multivariable analyses were performed to determine factors associated with *P. aeruginosa* carriage. Carriage rates differed significantly between cities ($p < 0.001$), and were lowest in Rome (healthy individuals 4.8%; patients 6.5%), followed by Rotterdam (healthy individuals 12.0%; patients 12.7%), and Jakarta (healthy individuals 28.6%; patients 24.0%). In carriers from Rotterdam, *P. aeruginosa* was most often detected in perianal swabs, while mostly in throat swabs among carriers from Rome and Jakarta. *P. aeruginosa* carriage had a seasonal association in patients from Rotterdam ($p = 0.014$) and Jakarta ($p = 0.020$). Among patients from Jakarta, female sex (aOR 1.98, 95% CI 1.02–3.84; $p = 0.045$) was associated with *P. aeruginosa* carriage. Overall, *P. aeruginosa* carriage rates and colonized body sites differ between cities and are likely associated with climate differences. Our findings warrant setting-specific adaptations of screening strategies and surveillance programs.**

Keywords Carrier state, Humans, Prevalence, *Pseudomonas aeruginosa*, Risk factors

Pseudomonas aeruginosa is a ubiquitous Gram-negative bacterium with a predilection for moist environments, and is capable of causing a range of severe and life-threatening infections, mainly in healthcare settings[1,2]. Community-acquired infections are less common, but may include infections of the ear, skin, and respiratory tract[1]. *P. aeruginosa* can be part of the microbial flora of healthy individuals, however, data on this are scarce[3]. Reported carriage rates in healthy individuals from the few available studies are between 1.2 and 15.8%, and mainly based on screening of the intestinal tract[4–10]. Other (moist) body sites may be colonized as well, though this has been studied even less. For instance, in the throat of healthy individuals from Indonesia and Vietnam, carriage rates of 1.2 and 1.8%, respectively, have been reported, and 5% in the navel of healthy individuals from the United States of America[7,8,10]. Furthermore, studies reporting on hospital admission screening for (multidrug-resistant) *P. aeruginosa* primarily involve critically ill patients, resulting in little available evidence on carriage rates in non-critically ill patients[11–17]. Despite screening more different body sites in studies with

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patients than in those with healthy individuals, body site-specific carriage rates are not provided and thus remain unknown. Besides the scarcity of evidence, the lack of uniformity in detection methods used across available studies further complicates the comparison and interpretation of overall and body site-specific carriage rates, both for healthy individuals and patients.

Evidence on risk factors associated with *P. aeruginosa* carriage is also limited, and even contradictory for healthy individuals. Whereas faecal carriage of *P. aeruginosa* appeared more likely in healthy individuals aged 60 years or older, another study found no association between age and *P. aeruginosa* colonization[4,8]. Regarding risk factors for patients, a significantly higher percentage of patients colonized with *P. aeruginosa* upon hospital admission were found to be 65 years or older and were more likely to have previously undergone surgery for gastrointestinal malignancy, compared to patients without *P. aeruginosa*[18].

To address these knowledge gaps, we aimed to determine *P. aeruginosa* carriage rates, overall and per body site (throat, navel, rectum), and associated risk factors for carriage among healthy individuals and patients upon hospital admission in three cities located in different climatic zones.

Methods

Study design and participants

This study is an extension of the SAMPAN study ("A Smart Surveillance Strategy for Carbapenem-resistant *Pseudomonas aeruginosa*"), of which the study design and methodology have been described elsewhere[19]. Briefly, the SAMPAN study is a cross-sectional study performed in three hospitals from countries with different climates: the Erasmus MC University Medical Center in Rotterdam, The Netherlands, with a temperate marine climate (cool summers and mild winters); Fondazione Policlinico Universitario Agostino Gemelli IRCCS in Rome, Italy, with a temperate Mediterranean climate (with dry, hot summers and rainy winters); and Dr. Cipto Mangunkusumo General Hospital in Jakarta, Indonesia, with a tropical monsoonal climate[19,20]. This study was conducted between August 2022 and June 2024 (Fig. 1). The study protocol was approved by the local Medical Ethics Review Committee of each participating hospital (Erasmus MC, MEC-2022–0014; Policlinico Gemelli, 0032025/22; Dr. Cipto Mangunkusumo, KET-17/UN2.FI/ETIK/PPM.00.02/2021) and was conducted in accordance with the principles of the Declaration of Helsinki. The SAMPAN study has been registered in ClinicalTrials.gov (registration number: NCT05282082). Written informed consent was obtained from all healthy individuals and patients enrolled in this study.

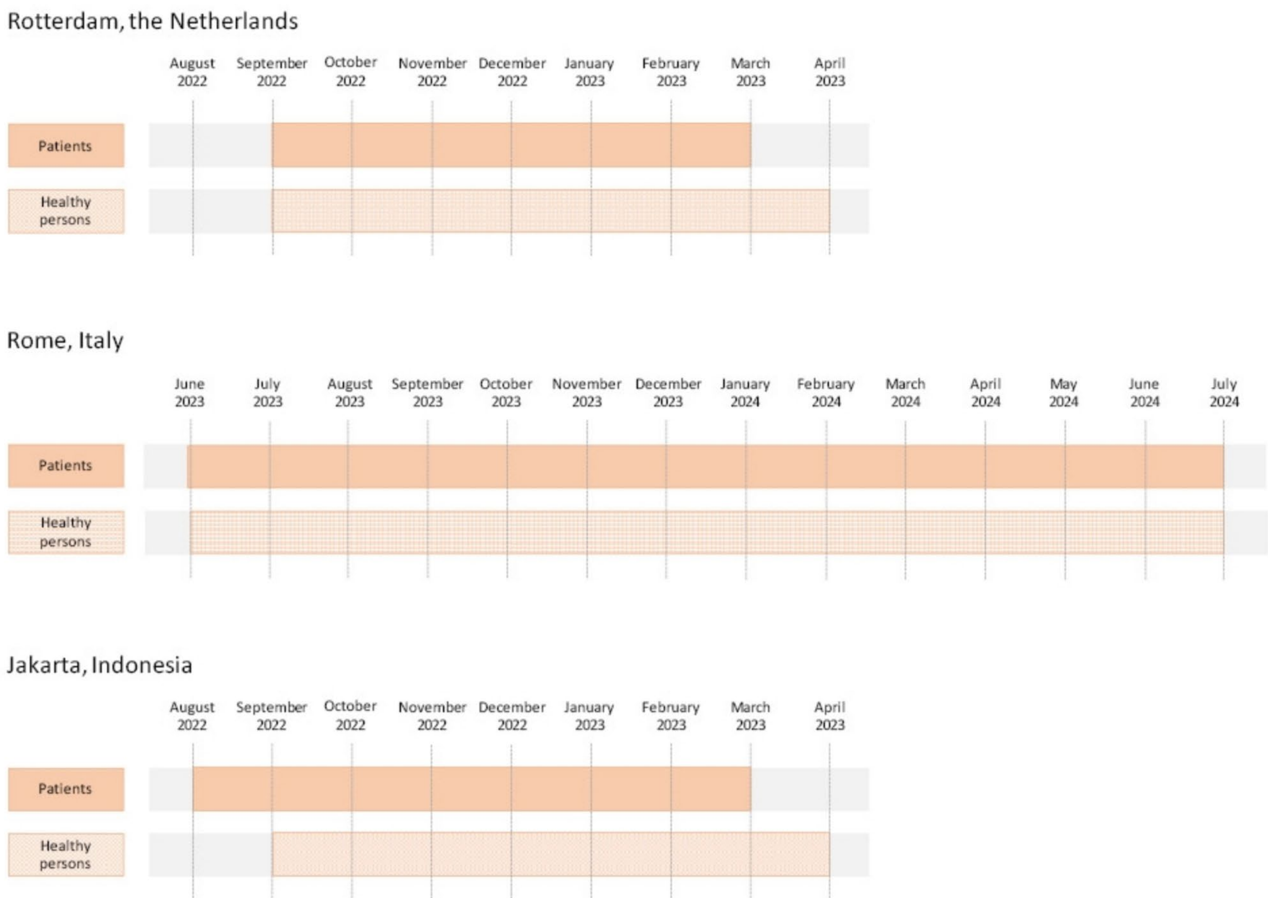

Fig. 1. Sampling period in the three participating sites.

Healthy individuals living in Rotterdam, Rome, or Jakarta, aged 18 years or older, were eligible for study participation. Recruitment strategies for healthy individuals have been described previously[19]. Additionally, patients admitted to medical or surgical wards were asked to take part in this study within the first 48 h of their admission to the hospital. Eligible patients were those aged 18 years or older, with an expected length of stay of ≥ 24 h, and who had a signed informed consent sheet. Patients with cystic fibrosis (CF) were excluded in this study. In Jakarta, patient enrolment was restricted to specific hospital buildings due to ethical clearance limitations, all of which had solely multiple-occupancy rooms. Both healthy individuals and patients were allowed to participate only once.

Participants were asked to provide throat, navel, and rectal/perianal samples, and to fill out a questionnaire on potential risk factors for *P. aeruginosa* carriage. The questionnaire consisted of 37 questions, with six general questions, 10 questions related to the individual's general health, three questions related to recent international travel, and 18 questions related to their contact with water[19]. Patients not able to provide answers to the questionnaire verbally or in writing, and healthy individuals and patients unable or unwilling to provide a rectal/perianal swab were also not eligible to participate in the study.

Microbiological methods

Throat, navel, and rectal/perianal swabs were collected using rayon swabs (Thermo Scientific™ Amies Agar Gel Transport swab, ThermoFisher Scientific, Breda, The Netherlands). Samples were enriched in 5 mL Tryptic Soy Broth (TSB) with 2 mg/L vancomycin and incubated overnight at 35 °C. From this broth, 10 µL was inoculated onto a *Pseudomonas*-selective agar plate, M-PA-C agar (BD Diagnostics, Breda, The Netherlands)[21]. Preliminary identification in Jakarta involved the oxidase test, followed by identification of all oxidase-positive isolates using the VITEK®2 with GN Identification Card (bioMérieux, Marcy L'Etoile, France). In Rotterdam and Rome, identification of presumptive *P. aeruginosa* colonies was performed using the Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight mass spectrometry (MALDI-TOF [Bruker Daltonics, Bremen, Germany])[19]. Antibiotic susceptibility was tested with the VITEK®2 in all cities, however, the AST-GN Card available in Indonesia does not contain imipenem in the panel. Therefore, additional disk diffusion was performed for both imipenem and meropenem in Jakarta. All *P. aeruginosa* isolates were stored at −80 °C.

Definitions

Carriage of *P. aeruginosa* was defined as having a positive screening culture in at least one of the three sampled body sites. To investigate the role of climatic factors on *P. aeruginosa* carriage, seasons were defined as winter (December-February), spring (March–May), summer (June–August), and autumn (September–November) for Rotterdam and Rome, and dry season (April–October) and monsoon season (November-March) for Jakarta.

Data collection

Basic patient characteristics, including age, gender, date of admission, and admission ward and specialization were collected from patients' (electronic) health records in each hospital[19]. Admission specializations were subsequently grouped into internal medicine and surgery (Supplementary File 1). Data from the questionnaires on patients' self-reported antibiotic use and national hospital admission(s) in the 3- and 12-months preceding study participation, respectively, were supplemented with data available from the electronic health record (EHR) in Rotterdam and Rome to minimize the potential effect of recall bias. In case a range was provided for the number of cigarettes per day and the number of times a person washed themselves per day/week, the average was used.

Data analysis

All analyses were performed with IBM SPSS (Version 28.0.1.0; IBM Corp., Armonk, New York, USA) and R (Version 4.4.2). Missing data are reported as such and were excluded from the analyses. Continuous variables are presented as medians with interquartile range (IQR) and categorical variables as absolute numbers and proportions. Chi-square and Kruskal–Wallis test were used to test for differences in basic characteristics (i.e., sex and age) of healthy individuals and newly hospitalized patients between the three cities. *P. aeruginosa* carriage rates, overall (both for healthy individuals and patients) and body site-specific (only for patients), were compared between the three cities using the Chi-square test. Carriers of *P. aeruginosa* were compared to non-carriers across a set of variables using Chi-square or Fisher's Exact Test (i.e., or the Fisher's-Freeman-Halton Exact Test for variables with more than two categories), and Mann–Whitney U Test, where appropriate. Post-hoc analyses were performed using pairwise comparisons with Bonferroni adjustment. Supplementary File 1 contains more detailed information on the data analyses with regard to the comparison between carriers and non-carriers of *P. aeruginosa*. To establish risk and protective factors of *P. aeruginosa* carriage (i.e., the dependent variable), we conducted multivariable logistic regressions with backward selection for patients from Rotterdam and Rome and for patients from Jakarta. Independent variables included in the multivariable logistic regression model were selected based on clinical relevance and results of the univariate analyses ($p < 0.1$), with sex and age included as standard parameters. Possible interactions were explored by comparing models with and without interaction terms using the likelihood ratio test. A p-value of < 0.05 was considered statistically significant.

Results

Healthy individuals

In Rotterdam, 2292 healthy individuals were invited to participate via an information letter. The outreach was subsequently broadened through social media, local newspapers, and by being featured on a local radio station (Fig. 2). In Rome and Jakarta, 550 and 704 healthy individuals were directly approached by inviting them to participate either at live events (e.g., during an open day at a university) or at their homes. Overall, 77 healthy

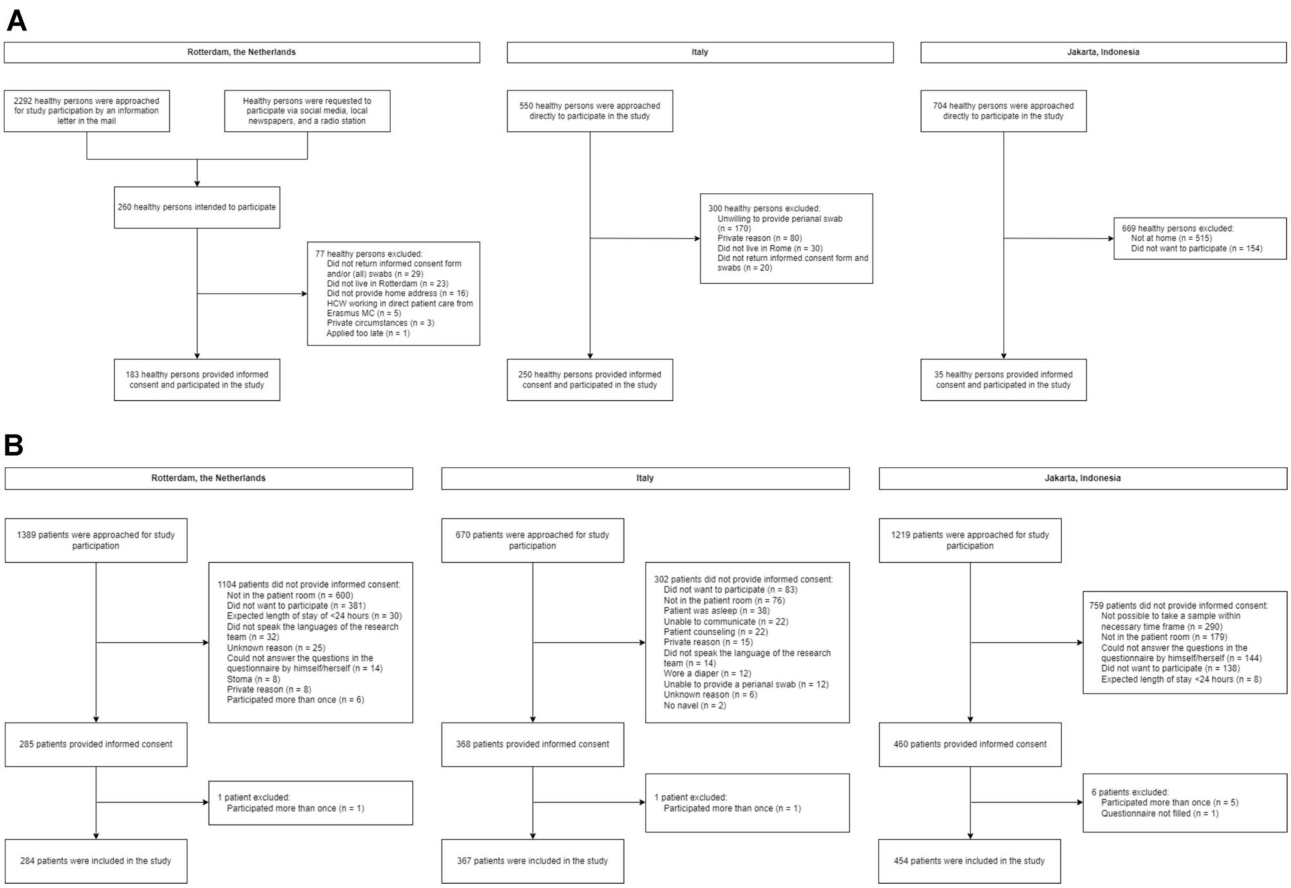

Fig. 2. Flowcharts of healthy individual and patient enrolment. (**A**) Healthy individuals. *HCW* healthcare worker. (**B**) Patients upon hospital admission.

individuals in Rotterdam, 300 in Rome, and 669 in Jakarta were excluded or unable to provide informed consent, resulting in a total of 183, 250, and 35 included healthy individuals in Rotterdam, Rome, and Jakarta, respectively (Fig. 2). Table 1 presents the demographic characteristics of enrolled healthy individuals. Although more female than male healthy individuals participated across the three cities (282 healthy women, 60.4%, versus 185 healthy men, 39.6%), there was no significant difference in sex among participants ($p = 0.074$). Furthermore, healthy individuals were youngest in Rome (median age 25 years, range 18–88 years), followed by Jakarta (median 51 years, 26–70 years), and Rotterdam (median 61 years, 21–87 years), with age differing significantly between cities ($p < 0.001$).

P. aeruginosa carriage was lowest in Rome at 4.8% (12/250 healthy individuals), followed by 12.0% (22/183 healthy individuals) in Rotterdam, and 28.6% (10/35 healthy individuals) in Jakarta, with carriage rates differing significantly between the three cities ($p < 0.001$) (Table 2). Carriage rates according to sex and age are presented in (Supplementary Table S1 and Fig. S1). Healthy individuals from Rotterdam were mostly found positive in perianal swabs (14/183 healthy individuals, 7.7%), whereas in healthy individuals from Rome and Jakarta, throat samples were primarily positive for *P. aeruginosa* (Rome: 7/250 healthy individuals, 2.8%; Jakarta: 6/35 healthy individuals, 17.1%). The detection rate of *P. aeruginosa* carriers was highest across all cities when screening both throat, navel, and rectum/perianal region (Supplementary Table S2). Supplementary File 3 provides MIC data of all *P. aeruginosa* isolates found in healthy individuals.

Health-related characteristics, data on recent travel, and exposure to water are shown in Supplementary Tables S3 and S4. Substantial differences in the populations from the different cities (e.g., in age) hampered between-city univariate analyses, therefore, only within-city analyses were performed to compare carriers and non-carriers of *P. aeruginosa*. Results from Rotterdam and Rome are presented in Supplementary Table S5, whereas the small sample size of healthy individuals in Jakarta prevented further analyses. In Rotterdam, carriers were more likely to be women ($p = 0.037$) and were significantly older than non-carriers (carriers: median age 67 years; non-carriers: median age 60 years; $p = 0.005$). Among healthy individuals from Rome, carriers did not differ significantly from non-carriers on any of the variables tested (Supplementary Table S5). Multivariate analyses were not performed for healthy individuals due to the overall low number of carriers.

Patients

A total of 1389 patients in Rotterdam, 670 patients in Rome, and 1219 patients in Jakarta were asked to participate within 48 h of admission. Ultimately, 284, 367, and 454 patients from Rotterdam, Rome, and Jakarta, respectively,

Characteristics	Rotterdam, The Netherlands (*N* = 183 healthy individuals)	Rome, Italy (*N* = 250 healthy individuals)	Jakarta, Indonesia (*N* = 35 healthy individuals)
	N (%)	*N* (%)	*N* (%)
Female sex	103 (56.6)[2]	152 (60.8)	27 (77.1)
Age, median (range)	61 (21–87)	25 (18–88)	51 (26–70)
Living situation			
In a healthcare facility	0 (0.0)	0 (0.0)	0 (0.0)
Independent	183 (100)	250 (100)	35 (100)
Median number of co-residents (IQR)	1 (1.0)[3]	2 (2.0)[4]	5 (3.0)
Paid/voluntary job		*N* = 249	
No	55 (30.1)	130 (52.2)	20 (57.1)
Yes	128 (69.9)	119 (47.8)	15 (42.9)
Contact with water during job for > 1 h/day[1]	6 (4.7)[2]	12 (10.2)[2]	7 (46.7)
Contact with animals more than 3x/week, more than 1 h/day	*N* = 182	*N* = 248	
No	134 (73.6)	175 (70.6)	33 (94.3)
Yes	48 (26.4)	73 (29.4)	2 (5.7)

Table 1. Basic characteristics of included healthy individuals. *IQR* interquartile range. [1]Respondents who reported being in contact with water for more than one hour per day as part of their job provided examples such as working at a cleaning company, washing dishes in a food stand, or working at an urban wastewater purification plant. [2]Missing in 1 healthy individual. [3]Missing in 12 healthy individuals. [4]Missing in 21 healthy individuals.

	Rotterdam, The Netherlands	Rome, Italy	Jakarta, Indonesia	*P-value*
	N (%)	*N* (%)	*N* (%)	
Patients	*N* = 284 patients	*N* = 367 patients	*N* = 454 patients	
Sampling site				
Throat	5 (1.8)[a]	17 (4.6)	65 (14.3)	< 0.001[f]
Navel	2 (0.8)[b]	6 (1.6)	29 (6.4)	< 0.001[g]
Rectum/perianal	32 (11.3)	8 (2.2)	31 (6.8)	< 0.001[h]
Total number of *P. aeruginosa* carriers	**36 (12.7)**[c]	**24 (6.5)**[d]	**109 (24.0)**[e]	< 0.001[i]
Healthy individuals	*N* = 183 healthy individuals	*N* = 250 healthy individuals	*N* = 35 healthy individuals	
Sampling site				
Throat	2 (1.1)	7 (2.8)	6 (17.1)	
Navel	6 (3.3)[j]	3 (1.2)	3 (8.6)	
Rectum/perianal	14 (7.7)	4 (1.6)	2 (5.7)	
Total number of *P. aeruginosa* carriers	**22 (12.0)**	**12 (4.8)**[k]	**10 (28.6)**[l]	< 0.001[i]

Table 2. Carriage rates, overall and per body site, of *P. aeruginosa* in patients and healthy individuals. [a]Missing in 2 patients. [b]Missing in 24 patients. [c]One patient was positive in both throat and perianal samples, and one patient in all three body sites. [d]Three patients were positive in both throat and navel samples, and four patients were positive in throat and perianal samples. [e]Five patients were positive in throat and navel samples, eight patients in throat and rectal samples, one patient in navel and rectal samples, and one patient in all three body sites. [f]Significant difference found in throat carriage between patients from Jakarta compared to Rotterdam and Rome. [g]Significant difference found in navel carriage between patients from Jakarta compared to Rotterdam and Rome. [h]Significant difference found in rectal/perianal carriage between patients from Rotterdam and Jakarta compared to Rome. [i]Significant difference found between the three cities, with all carriage rates differing significantly from each other. [j]Missing in 1 healthy individual. [k]One healthy individual was positive in both throat and perianal samples, and one healthy individual in navel and perianal samples. [l]One healthy individual was positive in both throat and rectal samples.

were enrolled (Fig. 2). Table 3 provides basic patient characteristics. Overall, significantly more male than female patients participated ($p < 0.001$), primarily in Jakarta (384 male patients, 84.6% versus 70 female patients, 15.4%). Patients differed significantly in age across the three cities ($p < 0.001$), with patients in Rome being the oldest (median age 64 years, range 19–96 years). The majority of patients in Rotterdam (*N* = 251, 88.4%) and all patients in Jakarta were admitted to a surgical ward, whereas in Rome, all patients were admitted to an internal medicine

Characteristics	Rotterdam, The Netherlands (*N* = 284 patients) *N* (%)	Rome, Italy (*N* = 367 patients) *N* (%)	Jakarta, Indonesia (*N* = 454 patients) *N* (%)
Female sex	115 (40.5)	174 (47.4)	70 (15.4)
Age, median (range)	63 (19–88)	64 (19–96)	44 (18–83)
Specialism			
Internal medicine	33 (11.6)	367 (100)	0 (0.0)
Surgery	251 (88.4)	0 (0.0)	454 (100)
Type of room			
Single-occupancy room	284 (100)	0 (0.0)	0 (0.0)
Multiple-occupancy room	0 (0.0)	367 (100)	454 (100)
Type of multiple-occupancy room		*N* = 366	
Two-person patient room	N/A	366 (100)	0 (0.0)
Four-person patient room	N/A	0 (0.0)	1 (0.2)
More than four-person patient room	N/A	0 (0.0)	453 (99.8)
Living situation			*N* = 453
In a healthcare facility	0 (0.0)	1 (0.3)	2 (0.4)
Independent	284 (100)	366 (99.7)	451 (99.6)
Median number of co-residents (IQR)	1 (1.0)[2]	1 (1.0)[3]	3 (2.0)[4]
Patient has a job (paid/voluntary)	*N* = 281		*N* = 453
No	165 (58.7)	192 (52.3)	252 (55.6)
Yes	116 (41.3)	175 (47.7)	201 (44.4)
Contact with water during job for > 1 h/day[1]	7 (6.0)	55 (31.4)	15 (7.5)[5]
Contact with animals (> 3x/week and > 1 h/day)	*N* = 278		*N* = 453
No	173 (62.2)	197 (53.7)	394 (87.0)
Yes	105 (37.8)	170 (46.3)	59 (13.0)

Table 3. Basic characteristics of patients upon hospital admission. *IQR* interquartile range, *N/A* not applicable. [1]Respondents who reported being in contact with water for more than one hour per day as part of their job provided examples such as washing dishes and cleaning as a barwoman and fishing for mussels at sea and farming oysters as a fisherman. [2]Missing in 57 patients. [3]Missing in 11 patients. [4]Missing in 24 patients. [5]Missing in 1 patient.

ward. Furthermore, patients in Rotterdam were exclusively admitted to single-occupancy rooms, while patients in Rome and Jakarta were solely admitted to multiple-occupancy rooms (Table 3).

Significant differences in carriage rates were observed between the three cities ($p < 0.001$), with *P. aeruginosa* carriage being 6.5% (24/367) in patients from Rome, 12.7% (36/284) in patients from Rotterdam, and 24.0% (109/454) in patients from Jakarta (Table 2). Supplementary Table S1 and Fig. S2 present carriage rates stratified by sex and age. *P. aeruginosa* was predominantly detected in perianal samples from patients in Rotterdam (32/284 patients, 11.3%), while mostly in throat samples from patients in Rome (17/367, 4.6%) and Jakarta (65/454, 14.3%) (Table 2). Significantly more *P. aeruginosa* carriage was observed in the throat ($p < 0.001$) and navel ($p < 0.001$) in patients from Jakarta compared to patients from Rotterdam and Rome. Also, rectal/perianal carriage of *P. aeruginosa* was significantly more often found in patients from Rotterdam and Jakarta compared to patients from Rome ($p < 0.001$; Table 2). The highest number of carriers was identified in each city by screening all three body sites (Supplementary Table S2). Supplementary File 3 contains MIC data of *P. aeruginosa* isolates found in patients from the three cities.

Supplementary Table S6 details health-related characteristics of enrolled patients, Table S7 patients' characteristics related to travel and exposure to water, and Table S8 within-city univariate analyses to compare *P. aeruginosa* carriers to non-carriers. Similar to the analyses for healthy individuals, between-city univariate analyses were not performed due to substantial underlying differences in the patient populations from the three cities.

Risk and protective factors for *P. aeruginosa* carriage

Univariate analyses revealed that *P. aeruginosa* carriage had a seasonal variation in Rotterdam ($p = 0.014$) and Jakarta ($p = 0.020$) (Supplementary Table S9). In Rotterdam, carriage of *P. aeruginosa* was more common in autumn compared to winter (22 carriers, 61.1%, in autumn; 14 carriers, 38.9%, in winter), while in Jakarta, *P. aeruginosa* carriage was more frequently detected in monsoon compared to dry season (67 carriers, 61.5% in monsoon season; 42 carriers, 38.5% in dry season) (Supplementary Table S9). The autumn and monsoon seasons were characterized by higher levels of precipitation compared to winter and dry season in Rotterdam and Jakarta, respectively (Supplementary Table S9). Similarly, multivariable analysis revealed increased odds of *P. aeruginosa* carriage in summer compared to winter (adjusted odds ratio (aOR) 6.38, 95% confidence interval (CI) 1.92–21.25; $p = 0.003$), followed by spring (aOR 5.58, 95% CI 1.64–18.92; $p = 0.006$) and autumn (aOR 2.49,

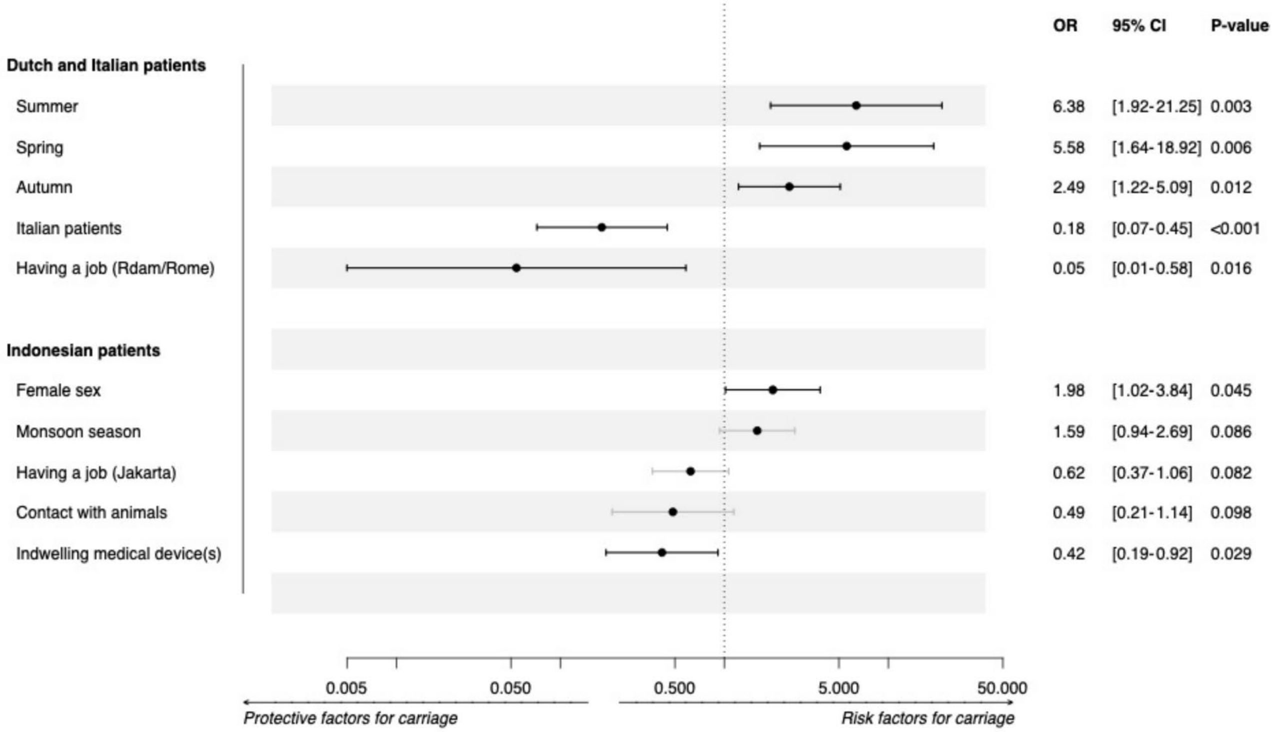

Fig. 3. Forest plot of risk factors for carriage of *Pseudomonas aeruginosa*[1]. [1]The findings for summer and spring are based solely on data from patients in Rome, as no corresponding data were available for patients in Rotterdam during these seasons.

95% CI 1.22–5.09; p = 0.012; Fig. 3); however, this finding is based solely on patients from Rome, as no summer and spring data were available from patients in Rotterdam. In addition, having a job (aOR 0.05, 95% CI 0.01–0.58; p = 0.016) and being admitted in Rome, as opposed to Rotterdam (aOR 0.18, 95% CI 0.07–0.45; p < 0.001) were identified as protective of *P. aeruginosa* carriage. Among patients from Jakarta, female sex (aOR 1.98, 95% CI 1.02–3.84; p = 0.045) was found to be associated with *P. aeruginosa* carriage, whereas having an indwelling medical device (aOR 0.42, 95% CI 0.19–0.92; p = 0.029) appeared protective of carriage (Fig. 3). The complete multivariable logistic regression models for Rotterdam/Rome and Jakarta are presented in Supplementary Tables S10 and S11.

Between-city descriptive analyses

Despite not conducting between-city comparative statistical analyses, several large differences related to exposure to water were observed. Firstly, patients drank bottled water much more frequently in Rome (60.4%) and Jakarta (63.9%) compared to patients in Rotterdam (30.3%), with *P. aeruginosa* carriers drinking bottled water more often than non-carriers in all cities (Rotterdam: 29.8% of non-carriers versus 33.3% of carriers; Rome: 59.7% of non-carriers versus 70.8% of carriers; Jakarta: 63.5% of non-carriers versus 65.1% of carriers; Supplementary Table S7 and Table S8). In Jakarta, untreated water was utilized both for drinking (28 patients, 6.2%) and food preparation (106 patients, 23.4%), which raises significant health concerns. On the other hand, Indonesian patients were also more likely to report using treatment processes (i.e., filtration or boiling) to make water safer to drink (Rotterdam: 26 patients, 9.2%; Rome: 26 patients, 7.1%; Jakarta: 139 patients, 30.6%). Secondly, patients from Rotterdam (267 patients, 94.3%) and Rome (353 patients, 96.4%) mainly washed themselves in the shower, while patients in Jakarta indicated that they most often used the bak gayung (356 patients, 79.5%). This is a basin that holds water for an extended period, from which water is scooped out using a bucket to pour it over the body for washing. Thirdly, western toilets were the most common type of toilet used in Rotterdam (279 patients, 98.9%) and Rome (367 patients, 100%), while in Jakarta these were mainly pit latrines (285 patients, 63.1%). These western toilets were primarily connected to piper sewer systems, which discharged into the municipal wastewater network. In contrast, pit latrines were reported to discharge mainly into septic tanks. Fourthly, washing clothes by hand was very uncommon in Rotterdam (1 patient, 0.4%) and Rome (3 patients, 0.8%), but was stated by approximately 20% of Indonesian patients as a common way to do laundry and was more often performed by *P. aeruginosa* carriers than non-carriers (71 non-carriers, 20.8% versus 25 carriers, 23.4%; Supplementary Table S7 and S8).

Discussion

This is the first study investigating *P. aeruginosa* carriage rates and associated risk factors among healthy individuals and patients upon hospital admission in three major cities located in different climatic zones. Carriage rates differed significantly between those cities and were lowest in Rome (4.8% in healthy individuals and 6.5% in patients), followed by Rotterdam (12.0% in healthy individuals and 12.7% in patients), and Jakarta (28.6% in healthy individuals and 24.0% in patients). Our findings highlight an important difference between carriers: while Dutch carriers were primarily positive in perianal swabs, *P. aeruginosa* was mostly detected in throat swabs in Italian and Indonesian carriers. Overall, the combination of throat, navel, and rectal/perianal swab resulted in the detection of the highest number of carriers in all three cities. Seasonality of *P. aeruginosa* carriage was observed in patients residing in a temperate marine climate (Rotterdam; between seasons autumn and winter) and tropical monsoonal climate (Jakarta), with more carriers identified in wetter periods of the year. Additionally, in Jakarta, female sex was found to be a risk factor for *P. aeruginosa* carriage.

To date, there are no recommendations for *P. aeruginosa* screening, and there is also a lack of evidence regarding the most sensitive body site or combination of body sites to detect *P. aeruginosa* colonization. Using a standardized approach across the three cities in this study, we were able to determine overall and throat, navel, and rectal/perianal carriage rates in healthy individuals and non-critically ill patients upon hospital admission. Carriage rates differed significantly between cities, and the predominant body site in which *P. aeruginosa* could be detected differed between humans from different cities. Our hypothesis is that differences in environmental conditions and lifestyle factors, including diet, have an effect on the body sites in which *P. aeruginosa* can be detected. For example, the use of bottled drinking water was much more common in Rome and Jakarta than in Rotterdam. However, several studies have shown that bottled drinking water may be a source of *P. aeruginosa*[22,23]. With the throat being the primary entry point for water during drinking, this may well become an important site for *P. aeruginosa* colonization. In addition, smoking was found to be much more common in healthy individuals and patients from Rome and Jakarta compared to Rotterdam, and also a higher percentage of carriers in Rome and Jakarta appeared to smoke. Previous research reported that smokers are more likely to become heavily colonized by Gram-negative bacteria in the oral cavity compared to non-smokers, which could be a potential explanation for Italian and Indonesian carriers being predominantly detected by throat swabs[24]. Although the combination of throat, navel, and rectal/perianal swabs resulted in the detection of the highest number of carriers in our study, it remains to be determined whether this is the most optimal combination. For instance, whether an individual is an intermittent carrier in the navel, caused for example by *P. aeruginosa* contaminated, dried-up water residue after washing, or truly reflects (persistent) carriage is unknown. Further explanatory research is needed to determine the most optimal combination of body sites and the underlying causes of differences in colonized body sites, as these offer insights to prevent further spread of *P. aeruginosa*.

Moreover, seasonal variations in the incidence rate of bacterial and viral infections are common. Several studies have reported such variations for *P. aeruginosa* (nosocomial) infections, with peaks in summer/warmer months of the year in various geographic regions[25]. In our study, seasonal variation of *P. aeruginosa* carriage was observed in the temperate marine climate, with higher carriage rates in autumn compared to winter. Although there was no significant seasonal variation in *P. aeruginosa* carriage in the temperate Mediterranean climate, the highest carriage rates were observed during summer and spring. Seasonal variation was also seen in the tropical monsoonal climate, with *P. aeruginosa* carriage being most common during monsoon season. In contrast to previous studies that linked seasonal variations in *P. aeruginosa* infections to increased temperatures, our findings seem to result from increased precipitation during certain months of the year in all three cities[25]. These findings have important implications for screening practices around the world, suggesting that increased awareness and surveillance for *P. aeruginosa* is needed during the wetter months of the year. However, further investigation across all four seasons in the temperate marine climate is warranted.

Sex and gender have increasingly been recognized as important determinants of disease, including infectious diseases[26]. Although sex-based differences in multidrug-resistant *P. aeruginosa* colonization at hospital admission have been investigated, its influence on *P. aeruginosa* colonization, regardless of antibiotic susceptibility, in humans is largely unexplored[27]. Interestingly, women were more likely than men to be colonized with *P. aeruginosa* in our study, and female sex was identified as a risk factor for *P. aeruginosa* carriage among Indonesian patients. A possible explanation for the predominant colonization of women, except among Dutch patients, could be that women often take on the majority of domestic duties, possibly resulting in increased frequency and duration of exposure to (contaminated) water sources prior to hospitalization. Additionally, Berglund et al. suggested that the differential use of cleaning products and cosmetics, usually more prevalent among women, might influence the presence of disinfectant resistant bacteria in men and women[28]. *P. aeruginosa* is intrinsically resistant to triclosan, commonly found in personal hygiene products, which could be a possible explanation for the higher carriage rates in women observed in this study. Despite collecting data on several variables related to water exposure and personal hygiene, participants were unfortunately not asked about their role in and frequency of domestic duties, and the use of cleaning products and cosmetics.

Knowledge on risk factors for acquisition of multidrug-resistant bacteria, e.g., carbapenem-resistant *P. aeruginosa* (CRPA), during hospitalization is quite extensive, yet there is a scarcity of evidence on risk and protective factors of *P. aeruginosa* carriage in healthy individuals and patients upon hospital admission. In our study, having a job was found to be a protective factor of *P. aeruginosa* carriage among Dutch and Italian patients. The most likely underlying explanation for this finding is the fact that, both in Rotterdam and Rome, there were relatively more carriers than non-carriers without a job, since carriers were also older than non-carriers (i.e., most individuals without a job were aged 60 years or older). Among Indonesian patients, having an indwelling medical device was identified as protective of *P. aeruginosa* carriage. However, having an indwelling medical device is commonly known as one of the leading risk factors for, for example, CRPA[29]. This finding is currently not understood, potentially being caused by sample selection without having any further clinical relevance.

Unfortunately, we were unable to determine risk factors and protective factors for *P. aeruginosa* carriage in healthy individuals. Further research is needed to determine those as well as to expand on our findings for newly hospitalized patients.

A strength of this study is the standardized approach, using homogeneous sampling (except for rectal swabs in Jakarta and perianal swabs in Rotterdam and Rome) and culture methods in the three participating cities, which improves the reliability and comparability of results. Furthermore, *P. aeruginosa* carriage was studied regardless of antibiotic susceptibility to gain a broader understanding of the body sites predominantly colonized by these bacteria, and not merely of those colonized by resistant isolates. In addition, this is the first study to compare overall and body site-specific carriage rates of *P. aeruginosa* in three cities located in different climatic zones, which also provided the opportunity to investigate seasonality of *P. aeruginosa* carriage.

This study also has potential limitations. First, due to unknown *P. aeruginosa* prevalences in the three countries, no sample size could be calculated prior to conducting this study. Therefore, the study may have been underpowered to determine certain effects, risk factors and/or protective factors. The observed *P. aeruginosa* prevalences offer guidance for future studies to confirm and expand on our findings. Second, differences in recruitment strategies may have introduced selection bias, which could have affected the observed *P. aeruginosa* carriage rates and hindered the between-city comparison of results. While we aimed to keep its risk to a minimum by using uniform recruitment strategies as much as possible (and permitted under ethical clearances), population differences were also influenced by other factors. For example, female patients in Jakarta were generally reluctant to participate in this study, because a rectal swab was required. This has led to a larger proportion of male patients participating in Jakarta. Third, the recruitment periods in Rotterdam and Jakarta were shorter than in Rome. This resulted, for example, in the inability to collect samples in spring and summer in Rotterdam. The highest odds of *P. aeruginosa* carriage observed during summer and spring, therefore, only reflect the findings from the Italian patients and should be further investigated in future studies. Fourth, only three body sites were screened in this study, which were selected based on evidence from previous research. Consequently, other relevant body sites may have been disregarded, potentially resulting in missed *P. aeruginosa* carriers. Further investigations could add to this knowledge by comparing the three screened body sites with additional body sites using the same standardized methods. Fifth, we took perianal instead of rectal swabs in Rotterdam and Rome due to restrictions in ethical clearance (i.e., unlike rectal swabs, perianal swabs are considered non-invasive methods). However, the sensitivity of perianal swabs is lower than that of rectal swabs[30]. To mitigate the likelihood of false-negative results, we developed and used a sensitive and highly selective culture method specifically targeted towards the detection *P. aeruginosa*[21]. Lastly, participants were asked to fill several questions on previous hospital admissions, antibiotic usage, and exposure to different sources of water in the months prior to study participation, which may have introduced recall bias. To minimize its potential effect, we supplemented self-reported data with data available from the EHR.

In conclusion, despite the ubiquitous presence of *P. aeruginosa*, its reservoirs in humans have been poorly studied to date. In our study, *P. aeruginosa* carriage rates and the body sites predominantly colonized were found to differ across cities and are likely associated with differences in climate. Our findings can be useful when designing surveillance strategies and warrant setting-specific adaptations.

Data availability

Metadata for human data are available in the data repository DataverseNL (https://doi.org/https://doi.org/10.34894/MZSJON). Data can be made available for reuse upon request through the corresponding author or the corresponding author's department (projectmanagement.mmiz@erasmusmc.nl).

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Author contributions
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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
The study protocol was approved by the local Medical Ethics Review Committee of each participating hospital (Erasmus MC, MEC-2022-0014; Policlinico Gemelli, 0032025/22; Dr. Cipto Mangunkusumo, KET-17/UN2.FI/ETIK/PPM.00.02/2021) and was conducted in accordance with the principles of the Declaration of Helsinki. The SAMPAN study has been registered in ClinicalTrials.gov (registration number: NCT05282082). Written informed consent was obtained from all healthy individuals and patients enrolled in this study.

Additional information
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