Mycoplasma pneumoniae infection in Yemen: incidence, presentation and antibiotic susceptibility

K.A. Al-Moyed[1] and H.A. Al-Shamahy[1]

عدوى المفطورة الرئوية في الجمهورية اليمنية: معدل الوقوعات، والمظاهر السريرية، والحساسية للمضادات الحيوية

ك. المؤيد، حسن الشماحي

الخلاصة: من أجل تحديد معدل الوقوعات والمظاهر السريرية (الإكلينيكية) والحساسية للمضادات الحيوية للمفطورة الرئوية *Mycoplasma pneumoniae* في المستشفيات الرئيسية بصنعاء، عاصمة الجمهورية اليمنية، قمنا بدراسة 405 مرضى تتراوح أعمارهم بين سن العاشرة والخامسة والستين ممن تم تشخيصهم سريرياً وبالتصوير الشعاعي لإصابتهم بعدوى الجهاز التنفسي السفلي. ويتم كشف الالتهاب الرئوي بالمفطورات بثلاثة طرق مختلفة: الزرع، واكتشاف المستضدات، وسيرولوجياً بكشف الغلوبولين المناعي من نوع الأيج - م IgM. أما الحساسية للمضادات الحيوية فيتم تحريها على المستفردات المؤكدة بطريقة التخفيف الكيروي للمرق. وقد وُجد من بينهم 125 مريضاً (30.9%) مصابين بعدوى حالية يغلب أن تكون التهاب القصبات والرئة ومعظمهم من الشباب. وكانت كل المستفردات التي تم اختبارها حساسة لكل المضادات الحيوية في المختبر مع كون الإريثروميسين هو الأكثر فعالية. وتدل النتائج على ضرورة استخدام أساليب مختلفة في التشخيص الروتيني لعدوى الالتهاب الرئوي بالمتفطرات في جمهورية اليمن بما في ذلك كشف المستضدات وكشف الغلوبولين المناعي من نوع الأيج — م سيرولوجياً.

ABSTRACT To determine the incidence, clinical presentation and antibiotic susceptibility of *Mycoplasma pneumoniae* at the main hospitals in Sana'a, we studied 405 patients clinically and radiographically diagnosed with lower respiratory tract infections aged 10–60 years. *M. pneumoniae* was identified by 3 different methods: culture, antigen detection and IgM serology. Antibiotic susceptibility testing was performed for confirmed isolates by macro-broth dilution technique. There were 125 patients (30.9%) with current infection, mostly among younger age groups, with bronchopneumonia the most common underlying clinical condition. All tested isolates were susceptible to all antibiotics in the *in vitro* antibiogram, with erythromycin the most active. The results indicate the need for different approaches in the diagnosis of *M. pneumoniae* infection in Yemen.

Infection à *Mycoplasma pneumoniae* au Yémen : incidence, tableau clinique et sensibilité aux antibiotiques

RESUME Afin de déterminer l'incidence, le tableau clinique et la sensibilité aux antibiotiques de *Mycoplasma pneumoniae* dans les principaux hôpitaux de Sanaa, nous avons procédé à une étude de 405 patients (fourchette d'âge : 10-60 ans) chez lesquels le diagnostic clinique et radiographique d'infection des voies respiratoires inférieures a été posé. *M. pneumoniae* a été identifié par trois méthodes différentes : culture, détection des antigènes et sérologie des IgM. Des tests de sensibilité aux antibiotiques ont été réalisés par la technique de macrodilution pour les isolats confirmés. Il y avait 125 patients (30,9 %) atteints d'une infection, la plupart dans les groupes d'âge les plus jeunes, la bronchopneumonie étant la condition clinique sous-jacente la plus courante. Tous les isolats testés étaient sensibles à l'ensemble des antibiotiques inclus dans l'antibiogramme in vitro, l'érythromycine étant le plus actif. Les résultats montrent la nécessité d'approches différentes pour le diagnostic de l'infection à *M. pneumoniae* au Yémen.

[1]*Department of Medical Microbiology, Faculty of Medicine and Health Sciences, University of Sana'a, Sana'a, Yemen.*
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Introduction

Mycoplasma pneumoniae infection has a worldwide distribution. It occurs throughout the year, most frequently in the colder months [*1,2*]. An estimated 11–15 million cases of *M. pneumoniae* infection occur annually [*1,3,4*]. *M. pneumoniae* is one of the most common etiologic agents of lower respiratory tract infections (RTI) among adults aged under 30 years, [*3,5–7*]. It causes an average of 15%–20% of all cases of community-acquired pneumonia in the general population [*1,8*] and up to 50% of pneumonias in closed populations, such as in schools and military institutions [*8,9*].

Clinical diagnosis of *M. pneumoniae* infection is frequently hindered by a lack of specific signs and symptoms [*10,11*], although in the majority of cases increasing cough, malaise, low-grade fever and headache are reported [*7,12*]. Timely laboratory diagnosis is also complicated. Culture of the organism is difficult and often only of retrospective value, as is the classic serologic assay, the complement fixation test [*11,13*].

Given the potential value of a rapid diagnostic method for *M. pneumoniae* infection, research has been focused in three areas: antigen detection, genetic probing with or without amplification and IgM serology (in instances where both antigen detection and direct genetic probing are limited by the quantity of organism available for detection, and a practical and accurate version of such methods is not widely available) [*14*]. Thus, rapid serologic methods based on the detection of immunoglobulin (Ig)M are the most amenable to practical use [*15*].

The drugs of choice for the treatment of *M. pneumoniae* infection are either erythromycin or tetracycline, the former being recommended for children due to the adverse effects of tetracycline on teeth [*16*].

Due to the difficulty in culturing *M. pneumoniae*, its slow growth rate, its sensitivity until now to macrolides and tetracyclines, and the lack of a readily-available method, antimicrobial susceptibility testing of *M. pneumoniae* is neither necessary nor appropriate for routine clinical microbiology laboratories and is usually performed only in larger institutions with special research interests [*3,17*]. The most widely used antimicrobial susceptibility tests to determine the minimal inhibitory concentration (MIC) of a drug in μg/mL for *M. pneumoniae* are the broth microdilution assay [*18,19*] and the agar dilution assay [*20*]. However, problems associated with these methods include the lack of a standard medium for test performance, discrepancies related to the pH required for optimal mycoplasmal growth versus optimal antibiotic activity and the lack of a standardized endpoint [*17*].

In the Republic of Yemen, as in other developing countries, acute RTIs are still one of the major causes of childhood morbidity and mortality. According to national epidemiology and disease surveillance data published by the Yemeni Ministry of Public Health in 1993 [*21*], the prevalence of acute RTI was 27% (23% among children aged 5–14 years). However, no epidemiological data has been available on the incidence or etiology of lower RTI by region in the Republic of Yemen. We therefore sought to estimate the prevalence of *M. pneumoniae* among children aged over 10 years and among younger adults diagnosed with lower RTI. Conventional and serological methods to detect *M. pneumoniae*, its antigen, and specific antibodies were used to determine the extent to which this organism is the causative agent in acute lower RTI. We also studied the clinical

picture of its infection and compared it with the clinical manifestations of other lower RTIs. Finally, we tested the susceptibility of *M. pneumoniae* to certain antibiotics commonly used in its treatment.

Methods

We studied all 405 subjects who had been clinically and radiographically diagnosed with lower RTI at hospital outpatient or inpatient departments during a 6-month period (October 1997–March 1998). The hospitals included were: Al-Kuwait University Hospital, Al-Jomhory Government Hospital, the National Tuberculosis Centre and the Central Health Laboratory, all in the city of Sana'a. The age of subjects ranged from 10–60 years.

Patients' complete histories were recorded onto a questionnaire. Details included name, age, sex, address, onset of illness, predisposing factors, presenting symptoms and signs, relevant history of respiratory diseases, antibiotic therapy, laboratory and clinical diagnosis.

Sputum and blood specimens were collected from every subject and transported to the laboratory as soon as possible (within 2 hours of collection) in sterile wide-mouthed, screw-capped containers. Samples were homogenized by adding an equal volume of dithiothreitol solution (10 mmol/L) [*22*], and divided into 2 portions: one for isolation of the organism, the other for mycoplasmal antigen detection after pretreatment with an equal volume of 2.5% skim milk buffer, and stored at –20 °C until tested. Blood (5 mL) was taken aseptically by venepuncture. After complete coagulation, the serum was separated, pipetted into Ependorff tubes and stored at –20 °C until tested for mycoplasmal IgM antibodies.

Isolation and identification of *M. pneumoniae* was performed according to Baron et al. [*23*]. Homogenized sputum (0.5 mL) was inoculated in a bottle of a commercially available mycoplasma selective diphasic culture medium (Oxoid Unipath Limited, Hampshire, England). The bottle was then loosely sealed and incubated in an anaerobic jar with 5%–10% carbon dioxide and very moist atmosphere at 35 °C for up to 4 weeks, and inspected daily for turbidity or colour change.

If, after 7 days, there was a change in the colour of the medium from violet (the original colour) to grades of green and finally to yellow, with no increase in turbidity, several drops of broth culture phase were subcultured onto a mycoplasma selective agar medium plate and incubated in the same atmosphere for another 7 days. The agar surface, observed for colonies after the fifth day under 10 × and 40 × magnification, had the appearance of fried eggs.

Definitive identification was performed for likely colonies by testing for haemolysis. After overlaying the agar surface with 5% sheep red blood cells in 1% agar, the plate was reincubated for 24 hours, and then examined for β-haemolysis around the *M. pneumoniae* colonies.

Antibiotic susceptibility testing of *M. pneumoniae* was performed according to Baron et al. [*23*] for the confirmed isolates by macro-broth dilution technique. Reference standard antibiotic powders were used (erythromycin, tetracycline, clindamycin and ciprofloxacin), obtained from their respective manufacturers (Sigma Chemical Company, St Louis, Missouri, United States of America (USA); Miles Laboratories, West Haven, Connecticut, USA) and the MIC of each drug in μg/mL was determined for *M. pneumoniae*.

M. pneumoniae antigen detection was performed according to Engvall and Perlmann [*24*], with some modifications. A test was developed in the Department of Medical Microbiology, Faculty of Medicine and Health Sciences, University of Sana'a, for the detection of *M. pneumoniae* whole antigen in sputum using indirect enzyme-linked immunosorbent assay test (ELISA). A commercially available ELISA kit (GenzymeVirotech GmbH, Rüsselsheim, Germany) was used for the detection of human IgM antibodies against *M. pneumoniae* in serum.

The questionnaire data were transcribed onto computer coding sheets, stored and statistically analysed using *Epi-Info*, version 6 (Centres for Disease Control and Prevention, Atlanta, Georgia, USA). The results were tested using the chi-squared (χ^2) test for significance.

Table 1 **Positive test results for *Mycoplasma pneumoniae* infection among 405 cases tested by different diagnostic methods**

Test results	Cases tested (n = 405)	
	No.	%
Positive culture and antigen		
Positive IgM	11	
Negative IgM	28	
Total	39	9.6
Negative culture, positive antigen		
Positive IgM	7	
Negative IgM	13	
Total	20	4.9
Negative culture and antigen		
Positive IgM	66	16.4
All negative	280	69.1

n = *total number of cases examined.*

Results

There were 405 patients diagnosed with lower RTI (167 outpatients, 238 inpatients), with a mean ± standard deviation age for all subjects of 32.4 ± 14.3 years. The mean age for males was 34.0 ± 13.8 years and for females, 30.6 ± 14.8 years.

Table 1 shows the distribution of the patients with RTI according to whether test results were positive or negative for *M. pneumoniae* by the different diagnostic methods (culture, antigen and IgM). *M. pneumoniae* colonies were isolated from 39 cases (9.6%). Antigen of *M. pneumoniae* in sputum was detected in 59 cases (14.6%). IgM antibodies against *M. pneumoniae* in serum were detected in 84 cases (20.7%). The total number of cases of recent *M. pneumoniae* infection was 125 (30.9% of all patients) when confirmation of disease was taken to be a minimum of a single positive result for one of the tests.

Table 2 shows the distribution of age, sex, and hospital category among all patients and among the 125 *M. pneumoniae* positive cases. Similar proportions of positive cases were found among males (31.8%) and females (29.8%). Significantly more schoolchildren (47.9%) tested positive ($P < 0.01$) and significantly fewer adults ($P < 0.01$). *M. pneumoniae* infection was also higher among inpatients ($P < 0.01$) and lower among outpatients ($P < 0.01$).

Of the 405 cases, 141 had lobar pneumonia, 77 had suspected tuberculosis (TB), 57 had bronchial asthma, 54 had chronic obstructive pulmonary disease (COPD), 52 had bronchitis and 24 had bronchopneumonia. Tables 3 shows the clinical diagnoses among the 125 *M. pneu-*

Table 2 **Age, sex and hospital category of the 405 patients examined and 125 cases testing positive for *M. pneumoniae***

Patient variable	Patients examined		Cases positive for *M. pneumoniae*		χ^2	*P*-value
	No.	%	No.	%		
Age group (years)						
10–18	73	18.0	35	28.0	12.20	< 0.01
19–24	75	18.5	23	18.4	0.00	NS
> 24	257	63.5	67	53.6	7.58	> 0.01
Sex						
Male	217	53.6	69	55.2	0.19	NS
Female	188	46.4	56	44.8	0.19	NS
Type of patient						
Outpatient	167	41.2	66	52.8	9.90	< 0.01
Inpatient	238	58.8	59	47.2	9.40	< 0.01
Total	405	100.0	125	30.9		

$\chi^2 \geq 3.84$; P < 0.05.
NS = not significant.

Table 3 **Clinical diagnosis among the 405 patients examined and the 125 cases testing positive for *M. pneumoniae***

Clinical diagnosis	Patients examined		Cases positive for *M. pneumoniae*		χ^2	*P*-value
	No.	%	No.	%		
Bronchopneumonia	24	5.9	10	41.7	1.40	NS
Lobar pneumonia	141	34.8	51	36.2	2.85	NS
Bronchial asthma	57	14.1	20	35.1	2.90	NS
Bronchitis	52	12.8	15	28.8	0.11	NS
Suspected tuberculosis[a]	77	19.0	18	23.4	2.50	NS
COPD	54	13.3	11	20.4	3.20	NS
Total	405	100.0	125	30.9		

$\chi^2 \geq 3.84$; P < 0.05.
[a]Clinical suspected cases of tuberculosis, but negative by laboratory methods (Ziehl-Neelsen stain plus culture).
COPD = chronic obstructive pulmonary disease.
NS = not significant.

moniae positive cases: 41.6% of cases with bronchopneumonia tested positive, 36.2% of lobar pneumonia cases, 35.1% of bronchial asthma cases, 28.8% of bronchitis cases, 23.4% of suspected TB cases and 20.4% of COPD cases. However, there was no statistical association between *M. pneumoniae* infection and patients' clinical diagnosis.

Table 4 shows the distribution of presenting signs and symptoms among the 125 cases testing positive: cough was the main presenting symptom in 95.2% of cases, followed by dyspnoea, chest pain, loss of appetite, low-grade fever, night sweating and haemoptysis. There was a statistically significant association between *M. pneumoniae* infection and the following presenting symptoms and signs: chest pain ($P < 0.05$), low-grade fever ($P < 0.01$) and night sweating ($P < 0.01$) (Table 4).

There were 18 cases (14.4%) with pulmonary complications among the 125 positive cases; 6.4% had respiratory distress, 5.6% had pleural effusion and 2.4% had lung abscess.

M. pneumoniae colonies were isolated from 39 cases (9.6%), 5 of which (from different isolation batches) were subjected to *in vitro* antibiogram testing. The tested isolates were found to be susceptible to erythromycin, tetracycline, clindamycin and ciprofloxacin, with erythromycin the most active antibiotic and ciprofloxacin the least active (Table 5).

Discussion

M. pneumoniae causes a wide spectrum of community-acquired respiratory infections, ranging from asymptomatic or mild upper RTIs to pneumonia. Although only 3%–10% of cases infected with *M. pneumoniae* actually develop pneumonia, the organism is the most common cause of primary atypical pneumonia and accounts for approximately 20% of all pneumonia cases in the general population [*25*].

The annual incidence of *M. pneumoniae* infection in the USA has been estimated to be almost 12 cases per 1000 inhabitants [*26*]. The infections associated with *M. pneumoniae* are usually endemic, although epidemics have been reported every 3–8 years. Reports of seasonal variations, usually with peak incidence during the winter months are not accepted by all researchers [*27*]. Reinfection may occur, and has been reported to be milder than primary infection, although, similarly, some findings do not support this [*28*].

In the present study, of the 405 cases clinically and radiographically diagnosed with lower RTI, *M. pneumoniae* was detected in 125 (30.9%), using one or more of three recent mycoplasmal infection markers: culture, antigen and/or Ig M serology. A similar detection rate was reported by Gray et al. in Djibouti (31%) [*29*]. Slightly higher rates were reported by Kleemola et al. (32.5%) in Finland [*9*], and by Chay et al. (33%) in Singapore [*30*]. By contrast, lower detection rates have been reported by Foy (15%) in the USA [*31*], and by El-Mofti (16%) and Hassan (18%) in Egypt [*32,33*].

This variation in incidence may be explained by the difference in geographical or climatic factors. Lower incidence rates have generally been reported in tropical regions and during warmer months, and higher rates in temperate regions and during colder months, although there is not universal agreement on this [*16,27,31*]. Other possible factors that may account for the variation in incidence rates include the socioeconomic and immune status of the patient, the hygiene status of the patient

Table 4 **Presenting symptoms and signs among the 405 patients examined and the 125 cases testing positive for *M. pneumoniae***

Presenting symptoms and signs	Patients examined		Cases positive for *M. pneumoniae*		χ^2	*P*-value
	No.	%	No.	%		
Cough	385	95.1	119	95.2	0.01	NS
Dyspnoea	324	80.0	104	83.2	1.16	NS
Chest pain	340	84.0	98	78.4	4.13	< 0.05
Low-grade fever	308	76.0	83	66.4	9.24	< 0.01
Night sweating	296	73.1	77	61.6	12.13	< 0.01
Loss of appetite	284	70.1	89	71.2	0.03	NS
Haemoptysis	150	37.0	44	35.2	0.26	NS

$\chi^2 \geq 3.84$; $P < 0.05$.
NS = not significant.

Table 5 **Minimal inhibitory concentrations of different antibiotics for *M. pneumoniae* isolates in the current study and comparisons with results of other studies**

Antibiotic	Minimal inhibitory concentration (µg/mL)			
	Current study		Kenny & Cartwright [*20*]	Waites et al. [*19*]
	Mean	Range	Range	Range
Erythromycin	0.03	0.008–0.062	0.031–0.062	< 0.008
Tetracycline	0.40	0.062–1.000	0.500–1.000	0.062–0.250
Clindamycin	0.50	0.125–1.000	–	< 0.008–0.500
Ciprofloxacin	1.20	0.500–2.000	2.000	1.000–2.000
WIN 57273	–[a]	–	0.031–0.125	–
Sparfloxacin	–	–	0.250–0.500	0.008–0.250
Ofloxacin	–	–	1.000–2.000	–
Lomefloxacin	–	–	2.000–4.000	–
Fleroxacin	–	–	4.000	–
Clinafloxacin (PD 127391)	–	–	–	< 0.008–0.031

[a]*Dashes denote values not reported.*

and patient's environment, and the methods used for detecting the etiological agent.

In the present study, school age children showed the highest proportion of positive results (47.9% of all 10–18-year-olds) compared with 30.6% of all young adults (19–24-year-olds) and 26.1% of adults (> 24 years old). This variation in detection rate was statistically significant for school age children and adults. Our finding that schoolchildren and young adults are the age groups most likely to be infected with *M. pneumoniae* agrees with what has been reported in the USA in 1973 and 1979 by Foy et al. [*31,34*], in Sweden by Vikerfors et al. [*10*] and in Denmark by Lind and Bentzon [*35*]. Schoolchildren and young adults may be more likely to be exposed to *M. pneumoniae* infection during this period of their lives because they spend much of this time in closed populations such as schools, universities and military camps.

In the present study, males had a slightly higher rate of infection with *M. pneumoniae* than females (31.2% and 29.8%, respectively), although the difference was not statistically significant. Similar results have been reported by others, although Foy [*31*] and Lind and Bentzon [*35*] suggest that mothers of schoolchildren are more likely to be at risk of *M. pneumoniae* infection because they are in closer contact with the infected child than is the father.

Bronchopneumonia was the most frequent underlying clinical condition among the positive cases in this study, with 41.6% of all bronchopneumonia cases in the study population testing positive. This was followed by lobar pneumonia, bronchial asthma and bronchitis. There was no statistically significant association between these clinical conditions and *M. pneumoniae* infection. Bronchopneumonia and lobar pneumonia were also the most frequent underlying clinical conditions among *M. pneumoniae* cases in Egypt as reported by El-Mofti [*32*]. Al-Rashed in Saudi Arabia also reported that bronchopneumonia, followed by lobar pneumonia, were the main clinical conditions among *M. pneumoniae* cases [*36*]. An association between *M. pneumoniae* infection and bronchial asthma has also been reported by Hassan in Egypt [*33*], although the author noted that further study was needed to confirm the association. Our findings regarding bronchitis were similar to those reported by Morguet et al. in Germany [*37*].

The most common presenting symptom among the 125 cases was cough, followed by dyspnoea, chest pain, loss of appetite, low-grade fever and night sweating. There was a statistically significant association between *M. pneumoniae* and each of the following symptoms: chest pain, low-grade fever and night sweats. Our findings regarding cough as the main symptom and low-grade fever agree with the results of others in Finland [*38*], Sweden [*10*], Norway [*39*] and the USA [*40*]. The other symptoms seen in the *M. pneumoniae* infected cases in our study are not supported by findings elsewhere. The main presenting symptoms vary considerably from study to study, reflecting the difficulty of clinical diagnosis of *M. pneumoniae* infection due to the lack of specific symptoms and signs [*7,11*].

In our study, pulmonary complications were identified in 14.4% of the 125 *M. pneumoniae* positive cases: respiratory distress, pleural effusion and lung abscess. Cassel and Cole [*8*], Fischman et al. [*41*] and Nagayama et al. [*42*] have previously noted the association between these complications and *M. pneumoniae* infection.

Antimicrobial susceptibility testing for *M. pneumoniae* is not routinely performed

in clinical microbiology laboratories. It is usually only carried out in larger institutions with special research interests, due to the difficulty in culturing the organism, its slow growth rate [*13,17*], its sensitivity until now to macrolides, quinolones and tetracyclines [*43*], and the lack of a readily-standardized method for testing the susceptibility [*13,17*]. In the present study, all the tested isolates were susceptible to erythromycin, tetracycline, clindamycin and ciprofloxacin, and the most active antibiotic was erythromycin. Similar results have been obtained by others [*19,20*]. In a study undertaken by Kenny and Cartwright, the *in vitro* susceptibility of *M. pneumoniae* was tested against several new quinolones, tetracycline and erythromycin [*20*]. The organism was found to be most susceptible to erythromycin, followed by WIN57273, sparfloxacin, tetracycline, ofloxacin, ciprofloxacin, lomefloxacin and fleroxacin. In another comparative study conducted by Waites et al., erythromycin was again found to be the most active antibiotic followed by PD 127391, sparfloxacin, clindamycin, tetracycline and ciprofloxacin [*19*].

Conclusion

The present study is the first to document the incidence, clinical picture and *in vitro* antibiotic susceptibility of *M. pneumoniae* among a sample of patients with lower RTI in the Republic of Yemen. Our findings suggest that in our country, *M. pneumoniae* infection is more common among school age children and young adults, and bronchopneumonia is the most common underlying clinical condition among patients with *M. pneumoniae* infection, an infection that cannot be distinguished from other respiratory infections on the basis of clinical and radiographic diagnosis alone. Despite the small size of tested isolates, erythromycin remains the drug of choice in the treatment of *M. pneumoniae* infection, although newer, more expensive antibiotics also show excellent effectiveness against the etiological agent.

Finally, our results indicate the need for different approaches in the routine diagnosis of *M. pneumoniae* infection in the Republic of Yemen, including antigen detection and IgM serology.

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Global atlas of infectious diseases

WHO's Communicable Disease Global Atlas (http://globalatlas.who.int/) brings together for analysis and comparison standardized data and statistics for infectious diseases at country, regional, and global levels. The analysis and interpretation of data are further supported through information on demography, socio-economic conditions and environmental factors. Key components of the Global Atlas include:

- Data query — allowing users to browse, view, query, search the contents of the WHO's communicable disease global database and output data in reports, charts and maps;
- Interactive mapping — providing a user-friendly mapping interface that allows selection of geographic areas of interest and creation of maps of diseases, and the location of health facilities, schools, roads, geographic features;
- Maps and resources — providing access to the public domain of static maps and related documents and statistics on infectious diseases.