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► **To cite this version:**

Hanen Smaoui, Hassiba Tali-Maamar, Saïd Zouhair, Selma Bouheraoua, Khaoula Mefteh, et al.. Implementation of a prospective study for enhancing surveillance of invasive bacterial infections in North Africa. *International Journal of Infectious Diseases*, 2022, 115, pp.101-105. 10.1016/j.ijid.2021.11.036 . hal-04133426

HAL Id: hal-04133426

<https://hal.science/hal-04133426>

Submitted on 22 Jun 2023

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ELSEVIER

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Implementation of a prospective study for enhancing surveillance of invasive bacterial infections in North Africa

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ARTICLE INFO

Article history:

Received 15 July 2021

Revised 12 November 2021

Accepted 23 November 2021

Keywords:

Invasive bacterial infections

real-time PCR

surveillance

Quality assurance

Algeria

Morocco

Tunisia

ABSTRACT

Objectives: We implemented a project named MENINGSTOP in three countries of North Africa (Algeria, Morocco and Tunisia). The main objective was to use real-time PCR to detect, identify and type the three main agents (*Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae*) responsible for invasive bacterial infections (IBI).

Methods: The protocol of WHO and US CDC for real-time PCR was used to detect and type the three bacterial agents in clinical samples. We also designated two quality exercises using an external interlaboratory study and cross-testing of 10% of randomly selected samples.

Results: Among the 752 samples tested, 18% were positive for one of the three agents. *N. meningitidis* was the most frequent globally reaching 9% of all samples (7% to 17% range) followed by *S. pneumoniae* 8% of all samples (6% to 15%). Group B meningococci was the most frequent (74% of all positive samples for meningococci and ranging from 50% to 90%). Quality assurance showed >85% correlation scores.

Conclusions: Real-time PCR can help improving epidemiological surveillance. Data confirm the prevalence of meningococci B. Our project adds a reliable tool to enhance surveillance and to help decision making in vaccination strategies against IBI.

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Introduction

Invasive bacterial infections (IBI) are responsible for high morbidity and mortality worldwide and remain a big challenge in public health. Three major agents: *Neisseria meningitidis* (Nm, meningococcus), *Streptococcus pneumoniae* (Sp, pneumococcus), and *Haemophilus influenzae* (Hi) are responsible for severe invasive infections in children >1 month of age.

Although meningitis is the most known form of IBI due to these bacteria, other clinical forms are reported such as septicemia but also bacteremic pneumonia and septic arthritis

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(Feldman and Anderson, 2019, Schaad, 1980). Evaluating the burden of IBI requires therefore better knowledge of all clinical forms and exhaustive testing. Nm, Sp, and Hi are capsulated bacteria with different polysaccharide capsular types that define serogroups (for Nm) and serotypes (for Hi and Sp). The distribution of these serogroups and serotypes varies significantly worldwide and over time. This information is crucial as vaccines already available against these three bacterial species target several but not all serogroups/serotypes. Decision making in vaccination strategies can benefit from representative data from active surveillance

Comprehensive data are still lacking for the three agents from many areas mainly due to lack of a global and easy-to-use diagnostic tests for IBI (Borrow et al., 2017, Ceyhan et al., 2012, Taha et al., 2020).

Molecular and nucleotide sequence-based diagnostic typing methods could be more widely deployed in clinical laboratories (Harrison et al., 2011). Culture-confirmed diagnosis is hindered by the failure to isolate bacteria because of their fragility or following an early antibiotic treatment but also due to difficulties in implementing conventional bacteriology laboratories. While PCR-based non-culture methods are now widely used to diagnose IBI, its implementation in many geographic regions is still lacking.

A call of action was recently adopted by the WHO, “Defeating meningitis by 2030”, which includes a roadmap that defines several pillars to achieve a vision of “a world free of meningitis” (WHO, 2020). Pillar 2 of the program was named “diagnosis and treatment” where laboratory confirmation is well defined for the main bacterial pathogens, with culture and real-time PCR being the gold standards. We aimed in this work to introduce standardized real-time-PCR method to detect Nm, Sp and Hi and to determine serogroups of Nm in three countries of North Africa (Algeria, Morocco and Tunisia) and to use it in particular to enhance the surveillance of invasive meningococcal disease. We describe here the organization and implementation of this project, named MENINGSTOP, and its first results.

Methods

Study sites

This was a prospective, observational, facility-based multicentric surveillance study based on a secondary use of samples withdrawn from routine case management of IBI, in 3 North African countries (Algeria, Morocco and Tunisia). In each country, specific hospitals were selected according to representativeness for the area, and an estimate of the catchment population of the site with 8, 4 and 8 hospitals in the three countries respectively. The location of study sites followed the demographic distribution in each country and covered about 50% in each country (Table 1). A coordinator laboratory was also designed in each of the 3 countries to perform real-time PCR for the project (The Institut Pasteur of Algeria, Marrakech University in Morocco, and Bechir Hamza children hospital at Tunis).

The implementation of the protocol was led in each country by the coordinator laboratory in 2019. The coordinator laboratories also conducted several local meetings to explain the protocol and the inclusion criteria. Trainings on the PCR protocol were conducted at the coordinator laboratory in each country as well as at Institut Pasteur, Paris, when required. Regular monthly meetings were conducted by virtual meeting to discuss progress and difficulties.

Patients and study population

The study population was composed of children between age of >1 month to <15 years who presented one of the following criteria:

Sudden onset of fever (> 38.5°C rectal or 38.0°C axillary) and at least one of the following signs: Neck stiffness and photophobia, bulging fontanels (in children < 2 years), altered consciousness with no other alternative diagnosis, characteristic non-blanching purpura, seizures (in infants 1–6 months old), and a combination of at least three of the following signs/symptoms (leg pain, arthralgia, abdominal pain, myalgia, lethargy, irritability, toxic appearance, cold extremities and delay in capillary refill time).

We included all patients at the study site with the defined symptoms who were provided (as well as their legal guardians) with information about the nature of the study and its procedures. Informed consent was obtained from legal guardians.

Actions to difficulties

Several difficulties were experienced during the early phase of implementation of the project and in particular:

- Obtaining the informed consent: We prepared written notes in Arabic for parents and for children who were able to read. The notes explained the project and emphasized that the project did not add any additional sampling but used the samples that were usually withdrawn for the routine management of IBI. The notes can be obtained by simple request to the corresponding author.
- Collecting and sending samples to the coordinator laboratories in the three countries. The participating hospitals in the three countries sent periodically the collected samples to the coordinator laboratories through a collecting network that used mainly road and/or postal transport. The immediate management of the patient was not delayed pending the result. However, the results of the real time PCR were communicated to clinicians as they could provide useful information to local health authorities in order to improve the healthcare system.
- Maintaining regular supply of materials (in particular enzymes and reagents): The primers, probes, controls, enzymes reagents and buffers were packaged as an “in house” kit by the Institut Pasteur, Paris and sent to the coordinator laboratories in the three countries 2–3 times per year to allow maintaining of the activities.

Laboratory procedures

Samples (cerebrospinal fluid [CSF], blood, skin biopsies, plural fluid and articular fluid) were taken according to the clinical presentations and as a part of routine management with no specific sample for the present study.

The required volume to perform the analysis was 200µl. Three PCR assays were performed for the presence of Sp, Hi and Nm with real-time PCR. The positive samples for *N. meningitidis* were then identified by serogroup-specific real time PCR. Those positive for *H. influenzae* were tested for serotype b. We used “group” instead of “serogroup” to indicate the grouping results of *N. meningitidis* as “serogroup” is a phenotype that is usually determined by agglutination (Fox et al., 2007).

Primers, probes and real-time PCR procedures were used to detect and type Nm, Sp, and Hi according to the detailed protocol in the WHO and CDC manual for laboratory methods for the diagnosis of meningitis caused the three bacterial agents targeted in this project (WHO, 2011). The three genes *sodC*, *lytA* and *hpd* were targeted to detect the three agents respectively.

When required, purification of DNA from clinical samples (recommended particularly for blood samples and skin biopsies) are performed using DNA (The High Pure PCR Template Preparation Kit, Roche). Results were listed according to values where each sample crosses the fluorescence threshold (Ct) that was higher

Table 1
Study sites

Country (Population)	Hospital	City/region	Population of the catchment area (% of the National Population)
Algeria (43 million)	University Hospital Nefissa Hamoud	Algiers	17.8%
	University Hospital Beni Messous		
	Hospital El Hadi Flici		
	University Hospital Batna	Batna	1.5%
	University Hospital Ben Boulaïd	Blida	1.7%
	University Hospital Constmatine	Constantine	1.9%
	University Hospital Oran	Oran	3%
Morocco (37 million)	University Hospital Mohamed Nedir	Tizi-Ouzou	2.6%
	University Hospital Ibn Rochd	Casablanca	20.3%
	University Hospital Ibn Sina	Rabat	13.5%
	Hospital Cheikh Zaid		
Tunisia (12 million)	University Hospital Hassan II	Fès	12.5%
	University Hospital Mohammed VI	Marrakech	13.4%
	Bécher Hamza Hospital	Tunis	22%
	University Hospital La Marsa		
	Military Hospital		
	University Hospital Bougatfa	Bizerte	5.4%
	University Hospital Fattouma Bourguiba	Monastir	5.4%
University Hospital Farhat Hached	Sousse	6.3%	
University Hospital Mahdia	Mahdia	4.2%	
University Hospital Nabeul	Nabeul	7.2%	

Table 2
Results of the external quality assurance study

Sample	Expected results	Lab 1	Lab 2	Lab 3
Sample 1	<i>Neisseria meningitidis</i> Non-groupeable	<i>Neisseria meningitidis</i>	Negative	<i>Neisseria meningitidis</i>
Sample 2	Negative	Negative	Negative	Negative
Sample 3	<i>Neisseria meningitidis</i> B	<i>Neisseria meningitidis</i> B	<i>Neisseria meningitidis</i>	<i>Neisseria meningitidis</i> B
Sample 4	<i>Haemophilus influenzae</i> b	Non tested	<i>Haemophilus influenzae</i> non-b	<i>Haemophilus influenzae</i> non-b
Sample 5	<i>Streptococcus pneumoniae</i>	<i>Streptococcus pneumoniae</i>	<i>Streptococcus pneumoniae</i>	<i>Streptococcus pneumoniae</i>
Sample 6	<i>Neisseria meningitidis</i> C	<i>Neisseria meningitidis</i> C	<i>Neisseria meningitidis</i>	<i>Neisseria meningitidis</i> C
Sample 7	<i>Neisseria meningitidis</i> W	<i>Neisseria meningitidis</i>	<i>Neisseria meningitidis</i>	<i>Neisseria meningitidis</i> W

than the negative controls and negative samples, and was within the start of the exponential phase for positive control.

Quality assurance

The performance of the PCR and the staff were monitored using two approaches of quality. The first one was conducted as an inter-laboratory external quality assurance study (EQA). After the implementation of the protocol, the Institut Pasteur, Paris distributed 7 samples to the three laboratories for identification and typing.

The second quality assurance study was conducted by retesting 10% of samples selected randomly from all samples in each country (the tenth sample in each laboratory), by the national reference center at the Institut Pasteur, Paris, France. The results were analyzed using Kappa Coefficient that calculated and expressed the agreement between two observers after considering random agreement (Steinijans et al., 1997).

Results

Implementation and quality assurance

The results obtained with the 7 EQA samples and the results of the testing by the three laboratories are depicted in Table 2 and were presented as a proportion of matching with the expected identification.

The correct species identification for the three agents was achieved for all the three laboratories with a score >85% of the expected species diagnosis. Negative samples were correctly identified as negative by all the laboratories. However, the grouping (prediction of serogroups and serotypes of Nm and Hi respectively)

was less optimal (Table 2). Several working sessions to discuss difficulties and improvement actions were organized.

The cross-testing of 10% of the samples from the three coordinator laboratories and the laboratory at the Institut Pasteur, Paris, France was performed using the same experimental procedures. The results were analysed by comparing the results of each laboratory to those obtained by the laboratory at the Institut Pasteur. As depicted in Table 3, Kappa coefficients were 0.65, 0.90 and 0.92 indicating substantial to excellent agreement.

Species and type distribution during the first year of the study

In the first year of the project, 82 samples (82 patients) were obtained from Algeria, 451 samples (451 patients) from Morocco, and 218 samples (from 175 patients) from Tunisia. Samples were mainly from CSF in the three countries and represented globally of 89.1% of all samples. However, blood, skin biopsies, and plural fluids were also obtained (Table 4). The proportion of positive samples for one of the three targeted agents ranged from 14% to 31%. Species distribution was close in the three countries. Nm and Sp were the most prevalent and represented 9% and 8% of all tested samples while Hi was much less frequently detected; it did not exceed 1% of all tested samples and it was absent in Morocco (Table 4).

Group B meningococci were the most prevalent group of Nm ranging between 50% and 90%. However, groups C and Y but not W were also detected in the three countries (Table 4).

Discussion

Data on the burden of invasive bacterial infections remain scarce from North Africa region. Information on country-wide

Table 3

Correlation between testing results obtained at the Institut Pasteur, Paris and each of the three participating laboratory

	Observed agreement	Chance agreement	Kappa coefficient	Level of the agreement
Lab1	0.95%	0.52	0.90	High
Lab 2	0.86%	0.61	0.65	Substantial
Lab 3	0.98%	0.68	0.92	High

Table 4

Data of the first year implementation of real-time PCR

	Algeria	Morocco	Tunisia	All
Number of samples (Number of patients)	83 (82)	451 (451)	218 (175)	752 (708)
Samples types % (N°)				
CSF	94% (78)	100% (451)	64% (141)	89.1% (670)
Blood	1.2% (1)		11% (24)	3.3 % (25)
CSF and blood	2.4% (2)		22% (48)	6.6% (50)
Skin biopsy	2.4% (2)			0.3% (2)
Pleural fluid			3% (5)	0.7% (5)
Number of positive samples % (N°)	31% (26)	14% (65)	20% (44)	18% (135)
<i>N. meningitidis</i>	17% (14)	7% (31)	9% (20)	9% (65)
<i>S. pneumoniae</i>	15% (12)	6% (28)	10% (22)	8% (62)
<i>H. influenzae</i>		1% (6)	1% (2)	1% (8)
Groups of <i>N. meningitidis</i> of all Nm				
B	50% (7)	90% (28)	65% (13)	74% (48)
C		3.2% (1)	10% (2)	4% (3)
Y	21% (3)	6.8% (2)	10% (2)	11% (7)
Non-determined	29% (4)		15% (3)	11% (7)

pneumococcal serotype distribution are still lacking, while reports described high prevalence of meningococci of group B (Brik et al., 2019, Razki et al., 2018, Tali-Maamar and Rahal, 2003). Vaccination against Hib and pneumococci are included in the national immunization schedules and/or available in through the private market in the three countries. However, these schedules do not currently include meningococcal vaccines Only MenACWY vaccination is required for pilgrims to Mecca, Saudi Arabia (Taha et al., 2020).

Improving diagnosis using PCR-based surveillance of IBI within this region would provide a more reliable burden estimate and would help establishing better fitted vaccination policies.

The MENINGSTOP project is in line with efforts to improve surveillance of IBI. This first report from the MENINGSTOP project showed successful implementation of real-time PCR as a routine tool for the management and surveillance of IBI in children. Quality assurance schemes were also implemented and should permit continuous improvement of the performance of the PCR testing in particular in grouping of Nm. The percentages of positive samples were similar to other studies (Heinsbroek et al., 2013). The first results showed low frequency of Hi positive samples that may be linked to the implementation of vaccination against Hi of serotype b in the three countries. The prevalence of Sp positive samples warrants the implementation of serotyping of Sp in order to better implement vaccination against this organism. These results also confirmed previous data on the prevalence of group B meningococci in North African countries (Brik et al., 2019, Razki et al., 2018, Tali-Maamar and Rahal, 2003). However, the recent emergence of SARS-CoV-2 and the global spread of COVID-19 prompted lockdowns worldwide with physical and social distancing. These measures seem also to impact invasive bacterial disease caused by Sp, Nm, and Hi that are known to be transmitted from person-to-person by air droplets. The number of positive samples for these agents is expected therefore to decrease as recently observed (Taha and Deghmane, 2020). The presence of group Y meningococci in children is worth exploring as this serogroup is more prevalent in immune deficient patients and in particular those with complement deficiencies (Rosain et al., 2017). Elaborating a clinical algorithm to explore complement deficiencies in such patients is warranted (El Sissy et al., 2019). The data that should be obtained by the end of this project will therefore be cru-

cial to better inform decision making in vaccination strategies in particular against Nm.

The major limitation of this phase of the MENINGSTOP project is that PCR assays are still performed in the coordinator laboratory. This may not impact on the immediate management of cases but can provide valuable data for global surveillance. The extension of PCR implementation to more laboratories should follow.

Conclusions

Real time PCR can be successfully used to enhance surveillance of IBI in North Africa and provide representative information to help decision making in vaccination strategies against IB

Contributions

Hanan Smaoui: Study design, Data collection, Data analysis, Writing.

Hassiba Tali-Maamar: Study design, Data collection, Data analysis, Writing.

Saïd zouhair Study: design, Data collection, Data analysis, Writing.

Selma Bouheraoua: Data collection, Data analysis.

Khaoula Meftah: Data collection, Data analysis.

Mohammed Bouskraoui: Study design, Data collection.

Amine Amiche: Study design.

Mouloud Khri: Study design.

Ala-Eddine Deghmane: Study design, Data collection, Data analysis, Writing.

Muhammed-Kheir Taha: Study design, Data collection, Data analysis, Writing.

Authors who are mentioned under "Study group" provided samples

Declaration of Competing Interest

This collaborative work was in part funded by Sanofi Pasteur but the latter was not responsible for data collection, data interpretation, and writing of the report. The corresponding author, MKT,

had full access to all the data in the study and had final responsibility for the decision to submit for publication. MKT also performs contract works for the Institut Pasteur funded by GSK and Pfizer outside the submitted manuscript, and MKT and AED have a patent with GSK, 630133. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject. AA and MK are Sanofi employees and may hold shares and/or stock options in the company.

Funding sources

This work was funded by Sanofi Pasteur and the Institut Pasteur.

Ethical approval

The Study is conducted in accordance with the ethical principles outlined in the Declaration of Helsinki (59th General Assembly of the World Medical Association, Seoul, Korea, October 2008) and in compliance with the latest local regulatory requirements. The protocol was approved and signed by the Scientific Leader, the coordinating investigator from each country (Algeria, Morocco, and Tunisia) as well as by the Institut Pasteur Paris. The study was approved by the Internal Regulatory Board of the Institut Pasteur as well as the national or local ethics committees from the three-participating countries.

Acknowledgments

We would like to thank the members of the National Reference Centre for Meningococci and *Haemophilus influenzae* at the Institut Pasteur for their technical help.

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