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Molecular epidemiology tools in the management of healthcare-associated infections: towards the definition of recommendations

L'epidemiologia molecolare nella gestione delle infezioni correlate all'assistenza: verso la definizione di raccomandazioni condivise

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Abstract

Introduction. Healthcare-Associated Infections (HAIs) are an important cause of morbidity and mortality worldwide and have a significant economic impact for health systems. Molecular epidemiology tools have a central role in HAI prevention programs. In order to give an overview of their specific advantages and disadvantages we reported current and new molecular typing methods for HAI outbreak detection and epidemiological surveillance.

Methods. The current review was drafted as a short version of a longer document written by the Public Health Genomics (GSP) working group, and the Italian Study Group of Hospital Hygiene (GISIO), entitled *Molecular epidemiology of Healthcare Associated Infections: recommendations from the Italian Society of Hygiene, Preventive Medicine and Public Health (SItI)*. This text considers various aspects related to HAIs: the role of genotyping and bioinformatics, the organizational levels of laboratories, as well as ethical and economic aspects.

Conclusions. The use of molecular epidemiology represents a key tool in the management of HAIs, to be used as a complement to conventional control measures. The present contribution aims to increase knowledge on the proper use of such methods, given the major challenge HAI represents for National Health systems.

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Key words: molecular epidemiology, healthcare-associated infections, genotyping methods

Riassunto

Introduzione. Le infezioni correlate all'assistenza (ICA) costituiscono un'importante causa di malattia e morte in tutto il mondo e hanno un notevole impatto sui costi sanitari. L'epidemiologia molecolare riveste un ruolo primario nei programmi di prevenzione e lotta alle ICA.

Metodi. Il presente contributo è ispirato dal lavoro di ricerca svolto per la stesura del documento *L'epidemiologia molecolare nelle Infezioni Correlate all'Assistenza: documento di indirizzo della Società Italiana di Igiene, Medicina Preventiva e Sanità Pubblica (SItI)*, a cura dei due gruppi di lavoro SItI Genomica in sanità pubblica (GSP) e Gruppo italiano studio igiene ospedaliera (GISIO); nel presente lavoro vengono considerati diversi aspetti legati alle ICA, dalla caratterizzazione molecolare al ruolo degli archivi bioinformatici, ai livelli organizzativi dei laboratori, agli aspetti socioeconomici.

Conclusioni. Il presente lavoro, focalizzato in particolar modo sulla realtà italiana, vuole contribuire alla sempre maggiore appropriatezza nell'utilizzo degli strumenti di prevenzione e controllo delle ICA, che costituiscono un'importante sfida per tutti i sistemi sanitari nazionali.

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Parole chiave: epidemiologia molecolare, infezioni correlate all'assistenza, metodi di genotipizzazione

OVERVIEW OF HEALTHCARE-ASSOCIATED INFECTIONS
Epidemiology of HAIs and antimicrobial resistance

Healthcare-Associated Infections (HAIs) represent a major issue of patient safety, associated with significant morbidity, mortality, and costs.[1] Epidemiological surveillance of HAIs is the cornerstone of effective infection prevention and control programs.[2] Currently, the coordination of the European surveillance of HAIs is carried out at the ECDC (European Centre for Disease Prevention and Control), where surveillance relies on the activities of three working groups: HAI-Net (Healthcare-Associated Infections Surveillance Network), EARS-Net (European Antimicrobial Resistance Surveillance Network), and ESAC-Net (European Surveillance of Antimicrobial Consumption Network).[3] In particular, the main priorities of HAI-Net are coordination of the surveillance of Surgical Site Infections (SSIs), the HAI surveillance in Intensive Care Units (ICUs), point prevalence survey of HAIs and antimicrobial use in acute care hospitals, and prevalence surveys of HAIs and antimicrobial use in European long-term care facilities.

Data from the ECDC point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals carried out in 2011-2012, which included 29 countries (231,459 patients), reported a HAI prevalence of 6%, ranging from 2.3% to 10.8%.[3] The most frequently reported were respiratory tract infections (pneumonia 19.4% and lower respiratory tract 4.1%), SSIs (19.6%), and urinary tract infections (19.0%). The microorganisms most frequently isolated from HAIs were *Escherichia coli* (15.9%), *Staphylococcus aureus* (12.3%), *Enterococcus spp.* (9.6%), *Pseudomonas aeruginosa* (8.9%), and *Klebsiella spp.* (8.7%). HAI prevalence was highest in patients admitted to ICUs (19.5%), with at least one HAI versus 5.2% of all other wards). Italy participated in the study with 14,784 surveyed patients; 6.3% of them had a HAI.[3] Pneumonia (21.6%) and other lower respiratory tract infections (2.4%) represented the most common type of HAI, followed by urinary tract infections (20.8%) and SSIs (16.2%), bloodstream infections (15.8%), and gastro-intestinal infections (6.6%). The most frequently isolated microorganisms were *E. coli* (13%), *K. pneumoniae* (11%), *P. aeruginosa* (11%), and *S. aureus* (8%).

As for incidence, two surveillance networks are active in Italy: SITIN (Sorveglianza italiana delle infezioni in terapia intensiva, Italian surveillance of infections in ICUs) which collects data from three surveillance Italian systems (GiViTI, Gruppo italiano per la valutazione degli interventi in terapia intensiva; SPIN-UTI, Sorveglianza prospettica delle infezioni nosocomiali nelle unità di terapia intensiva; and SITIER, Sorveglianza delle infezioni in terapia intensiva dell'Emilia-Romagna) and SNICh (Sistema nazionale di sorveglianza delle infezioni del sito chirurgico). Data from the last SITIN report showed an incidence of bloodstream infections of 4.2/1,000 patient-days in 2011, and 4.6 in 2012; incidence of urinary tract infections in 2011 and 2012 was respectively, 1.9 and 2.7/1,000 patient-days, while incidence of pneumonia was, respectively, 5.8 e 5.4/1,000 patient-days.[4]

In 2011, SNICh registered an SSI incidence of 1.3% for or-thopaedic procedures, and 1.7% for non orthopaedic procedures.[5]

Recent years have seen a dramatic increase in HAIs caused by antibiotic-resistant bacteria, leading to extra healthcare costs, treatment failure, and deaths.[6] In general, lower percentages for resistance are reported by countries in the north and higher percentages are reported by countries in the south and east of Europe. In the period 2010-2013, in Italy, as in the rest of Europe, there was an increase in combined *K. pneumoniae* resistance (fluoroquinolones, aminoglycosides and 3rd generation cephalosporins) from 26.3% to 41.8%; in particular, carbapenem-resistant *K. pneumoniae* showed an alarming increase in 2013, reaching 34.3% compared to 15.2% in 2010. Unlike the decreasing European trend, the percentage of meticillin-resistant *S. aureus* remained constant over this four-year period, with a value exceeding 30%. Generally speaking, antimicrobial resistance in Italy reached percentages that are from two (3rd-generation cephalosporin resistant and fluoroquinolone-resistant *E. coli*, piperacillin-tazobactam resistant *P. aeruginosa*) to four times (carbapenem-resistant *K. pneumoniae*) higher than the European average.

Socioeconomic impact of HAIs

The relevant HAI-related resource consumption has the potential to inflict significant economic consequences on national healthcare systems.[7] Analysis of the socio-economic impact of HAIs includes several cost categories, such as direct costs (e.g., costs of instruments, equipment, therapy, days of hospitalization), indirect costs (morbidity, mortality, loss of productivity and profit) and intangible ones (e.g., psychological damage, pain, loss of social role).

Direct costs are usually paid by the healthcare system, while indirect and intangible ones are often iborne by patients. The CDC (Centers for Disease Control and Prevention) of Atlanta calculate that, given the range of effectiveness of possible infection control interventions, the benefits of HAI prevention range from a low of $5.7 to $6.8 billion to a high of $25.0 to $31.5 billion per year.[7] Comparing these costs with those for other important diseases such as stroke (about $6.7 billion/year), or chronic obstructive pulmonary disease ($4.2 billion), it is worth considering an investment in HAI prevention, given its cost effectiveness.[7,8] An overview on literature reporting on the economic impact of HAI can be found in table S1 (see supplementary material online). Two main categories of studies are represented: the first includes studies that estimate the cost of HAIs at different levels, e.g., for the National Health System, the insurance system, and a single hospital, while the second is about retrospective analyses of the cost of a single HAI, or the costs of a HAI outbreak.

MOLECULAR EPIDEMIOLOGY TOOLS
Molecular typing methods

Molecular typing methods are used in different fields to study the genomic organization and evolution of microorganisms, to discern and track individual strains and to identify patterns of infection and sources of transmission. Particularly, such tools are

Molecular typing methods	Advantages	Limits	Applications and notes
Pulsed-Field Gel Electrophoresis (PFGE)	■ excellent discriminatory power ■ intra- and inter-laboratory reproducibility ■ high epidemiological concordance ■ moderate cost	■ lacking in ease of use and speed ■ low resolution for similar fragment size	■ useful for outbreak investigation and epidemiological surveillance, at local or large-scale level ■ international databases are widely available
Amplified Fragment Length Polymorphism (AFLP)	■ excellent discriminatory power	■ lacking in ease of use ■ high cost	■ as PFGE
Random Amplification of Polymorphic DNA (RAPD) and Arbitrarily Primed Polymerase Chain Reaction (AP-PCR)	■ rapidity and ease of use ■ low cost	■ low discriminatory power ■ low intra-laboratory reproducibility	■ extensively adopted for outbreak investigation
Repetitive-element Polymerase Chain Reaction (rep-PCR)	■ high rapidity and discriminatory power for some microbial species ■ low cost	■ poor reproducibility	■ semi-automatic methods adopted for outbreak investigation and epidemiological surveillance at local level ■ not recommended for large-scale studies
Variable-Number Tandem Repeat (VNTR) typing	■ excellent reproducibility ■ ease of use ■ accessibility ■ rapidity ■ moderate cost	■ moderate inter-laboratory reproducibility	■ multilocus VNTR analysis (MLVA) is adopted for outbreak investigation, but is not validated in epidemiological surveillance networks
Single Locus Sequence Typing (SLST)	■ high discriminatory power for some species (e.g. *spa*-typing for *S. aureus*, ■ ease of use ■ rapidity ■ moderate cost	■ potential misclassification of particular types, due to recombination and/or homoplasy	■ used to determine the relationships among bacterial isolates for outbreak investigation and epidemiological surveillance at local and international level
Multilocus Sequence Typing (MLST)	■ excellent reproducibility	■ lacking in ease of use and not speed ■ limited accessibility ■ high cost ■ poor discriminatory power for some species	■ used for large-scale epidemiological studies and for performing bacterial population genetics studies ■ not suitable for local outbreak investigation and surveillance studies
Comparative Genomic Hybridisation (CGH): microarrays	■ high throughput technique	■ the intra- and inter-laboratory reproducibility of microarray data needs to be established prior to the application ■ high cost ■ poor accessibility ■ do not allow the identification of sequences which are not included in the array ■ not yet suited for day-to-day clinical application	■ used in real-time outbreak investigations ■ rapid and microarray assays for diagnostic testing and epidemiological investigations have been developed ■ adopted for simultaneous genotyping and profiling to determine antimicrobial resistance and virulence potential
Optical mapping	■ high accuracy ■ high discriminatory power	■ high cost ■ poor accessibility	■ successfully used in retrospective outbreak investigations to examine the genetic relationships among bacterial isolates (e.g., *E. coli* O104:H4)
Whole Genome Sequencing (WGS)	■ high throughput technique	■ lacking in ease of use ■ limited accessibility	■ accurate identification and characterization of bacterial isolates ■ in the near future WGS will become a highly powerful tool for outbreak investigations and surveillance schemes in routine clinical practice

Table 1. Characteristics of the main molecular typing methods (ref. 9-11). / **Tabella 1.** Caratteristiche dei principali metodi di genotipizzazione (ref. 9-11).

used in the epidemiological surveillance of infectious diseases, including HAIs, and for outbreak investigation.[9,10]
Over the last years different molecular typing methods have been implemented worldwide and guidelines and general criteria have been proposed to interpret the obtained results.[10,11]
However, typing methods need to be evaluated and validated with respect to a number of performance and convenience criteria.[9,10] The performance criteria, based on intrinsic features of a method, mainly refer to the stability and reproducibility of the marker assessed, providing universal typeability of all isolates. In addition, feasibility, time, cost, and portability, representing the convenience criteria, need to be considered.[10] The latter criterion is of great importance, especially in large-scale surveillance, as the use of electronic databases allows microbiologists and public health institutions to monitor the spread of strains beyond the hospital/local level.

Finally, since analyses may depend on different interpretations and have different requirements, there is no universally applicable typing method. Nevertheless, the increasing need to communicate among laboratories and to exchange outbreak investigation or surveillance data requires a high degree of agreement on common methods. Such standardization is gradually being undertaken for the most popular and reliable typing methods. An important thematic issue published by *Eurosurveillance* in January 2013 provided a series of reviews and original research articles that evaluated the latest progress in molecular epidemiology strategies and tools, and illustrated their applications in public health. Following this document, the Public Health Genomics (GSP) working group and the Italian Study Group of Hospital Hygiene (GISIO), of the Italian Society of Hygiene, Preventive Medicine and Public Health (SItI), wrote a collaborative document, entitled *Molecular epidemiology of healthcare associated infections: recommendations from the Italian Society of Hygiene, Preventive Medicine and Public Health (SItI)*.[12,13] This text has been drafted as a short version.

The previously mentioned *Eurosurveillance* issue included a review,[11] published on behalf of the ESCMID Study Group of Epidemiological Markers (ESGEM), conducted using the PubMed database with a combination of specific keywords to retrieve the relevant articles. Table 1 reports the characteristics, advantages, and limits of the main molecular typing methods for the management of HAIs based on that review.

Bioinformatics and surveillance networks in bacterial molecular epidemiology

The development of DNA sequencing methods and other molecular methodologies has led to substantial improvement in microorganism identification, which is accompanied by generation of large amounts of data. Bioinformatics answered to the need to develop tools to store and analyze such a plethora of information, through dedicated algorithms for data sharing and integration, image analysis, and mining. Online databases for microbial typing data, analytic software for gel-based molecular typing techniques, and models for phylogenetic inference analysis have been developed, and this has made it possible for bacterial typing to extend beyond outbreak investigation at the local level.[14] The ability to share information using the Internet has led to the evolution of specialized software in which nationwide or worldwide comparisons can be performed. PulseNet represents the forerunner of molecular subtyping networks, based on standardized PFGE protocols for the identification of pathogenic food-borne bacteria. Gradually, online database and networks for the other typing methods have grown and are continuously updated. Thanks to the portability of sequence data, Multilocus Sequence Typing (MLST) websites and databases are extensively and successfully adopted for nomenclature purposes, allowing disambiguation when analyzing and comparing results and to infer patterns of phylogenetic descent through comparison of allelic profiles.[15] Sequence-based typing methods have led to the development of new analysis methodologies, such as eBURST or goeBURST, which have made it possible to determine the genetic

population structure of many bacterial species, bacterial evolution and spread.[16] More sophisticated software has been developed to handle the ever larger amount of data generated by Next Generation Sequencing (NGS), and is slowly but successfully being applied to a variety of public health problems, ranging from outbreak investigations to long-term epidemiologic studies.

Finally, integration of genomic and epidemiological databases with NGS data will be the next frontier in bacterial epidemiology, in order to empower stakeholders in everyday public health decisions. As the spatial component in epidemiological studies is of great importance, the ability to monitor the geographic spread of clones at different levels can provide a comprehensive perspective of the dissemination of successful clones. Through the combined analysis of this information, the broader use of geographic information in phylo-geographical approaches will allow a better understanding of the spread of particular clones. Once NGS becomes widely adopted, the public health community challenges will be to define standards for analysis and reporting, in order to produce the desired reproducibility, and a common language for typing based on NGS to be useful in clinical settings.[14]

MOLECULAR EPIDEMIOLOGY IN THE MANAGEMENT OF HAIs
Role of molecular epidemiology in infection prevention and control programs

Molecular epidemiology for the characterization of microorganisms represents an essential component in the surveillance of infectious diseases and outbreak investigations, especially for the prevention and control of HAIs.[10] Furthermore, molecular typing methods have been successfully used to study markers associated with pathogenicity and antibiotic resistance. This approach has proven particularly useful over the last decade, as multidrug-resistant microorganisms have been implicated in severe HAIs and their occurrence has increased gradually, which has also made it difficult to control outbreaks.[17-19]

Generally, typing methods can be described as comparative or library typing. In comparative typing, which is mainly used for outbreak investigation, outbreak-related and unrelated isolates are tested to address infection control strategies. In the long term, comparison between outbreak related isolates with isolates from the past or future is not relevant. On the contrary, library typing methods are useful to compare strains from a current outbreak with previous circulating strains and can be used in different laboratories at various time intervals, in order to generate data to be aggregated in a single database for comparative assessment in great detail at any time.[9,10,20]

Molecular characterization can be used to study the genetic relationships between isolates and identify their mode of transmission (person to person, with environmental surfaces or invasive devices). Besides, it allows to identify episodes of intra- or inter-hospital transmission, or in other healthcare settings, to design appropriate control measures to contain the epidemic spreads of isolates associated with colonization and/or HAI episodes.[17-19] A molecular typing approach for HAI control

provides an insight into the population dynamics of microorganism circulation in order to allow the design of evidence-based strategies to decrease infection rates to the irreducible minimum.[17]

In order to improve collaboration between expert and reference laboratories to support prevention and control strategies, the ECDC facilitates, at the European level, interdisciplinary collaboration and the assessment of public health needs for the integration of molecular typing data into surveillance and epidemic investigations.[21]

In view of this premise, the central role that molecular epidemiology might gain in the management of HAIs is evident, both in dissecting the clonality among strains, thus confirming or denying the presence of pseudo-outbreaks, and showing the effectiveness and cost-effectiveness of HAI prevention and control programs.[22] Understanding the clonality between microorganisms can allow to quickly establish the degree of relationship between them, thus resulting in a more accurate and rapid implementation of preventive measures or, in the case of absence of clonality, helps to avoid expensive measures of caution that are necessary when awaiting traditional investigations.[22]

Ethical, legal and social aspects (ELSI)

The outstanding diagnostic potential of genotyping techniques in detecting the origin and routes of transmission of an outbreak raises many questions from an ethical point of view. These issues concern different aspects such as the right to privacy, respect for individual autonomy, informed consent, and moral obligation on the part of every individual to avoid the spread of a contagious disease.[23,24]

Given the unique features of molecular techniques it would be necessary to implement a regulatory framework under which their use may be applied in public health practice. Molecular epidemiology for bacterial genotyping, as well as human DNA genotyping, is, in fact, gaining an increasing important role in court cases and legal affairs in order to help the process of attribution of responsibility. Nevertheless, it is crucial for every professional figure involved in the use of microbial forensics to be well aware that molecular epidemiology can in no-way be considered as a substitute for a thorough epidemiological investigation that can integrate molecular methods with the other traditional epidemiological tools.[23] It must be clear that the use of molecular epidemiology without the contribution of other tools and professional figures such as hygienists or epidemiologists, could be insufficient to handle a case of causal attribution of responsibilities, thus resulting in negative consequences for the community and risks – such as stigmatization, penalties, economic risks – for the individuals involved.

CONCLUSIONS

Patient safety and quality of care have now gained the top of the political agenda of the European Union. Council Recommendation 2009/C 151/01 proposes an overarching EU-level strategy to promote patient safety and underlining the importance of correct management of HAIs as a cornerstone to achieve safety and a high quality healthcare delivery.[25] Proper education and training of health professionals on these topics is considered of crucial importance to achieve these goals.

This narrative review is based on the research made for the drafting of *L'epidemiologia molecolare nelle infezioni correlate all'assistenza: documento di indirizzo della Società Italiana di Igiene, Medicina Preventiva e Sanità Pubblica (SItI)*,[12] a product of the joint effort of two different working groups of the Italian Society of Hygiene, Preventive Medicine and Public Health. These two documents may be considered a step toward the implementation of knowledge and education on HAI prevention and management in Italy, in line with the European recommendations.[13]

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