

R E V I E W

Molecular epidemiology of antimicrobial resistant microorganisms in the 21th century: a review of the literature

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Summary. Healthcare-associated infections (HAIs) are the most frequent and severe complication acquired in healthcare settings with high impact in terms of morbidity, mortality and costs. Many bacteria could be implicated in these infections, but, especially multidrug resistance bacteria could play an important role. Many microbial typing technologies have been developed until to the the bacterial whole-genome sequencing and the choice of a molecular typing method therefore will depend on the skill level and resources of the laboratory and the aim and scale of the investigation. In several studies the molecular investigation of pathogens involved in HAIs was performed with many microorganisms identified as causative agents such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Clostridium difficile*, *Acinetobacter* spp., *Enterobacter* spp., *Enterococcus* spp., *Staphylococcus aureus* and several more minor species. Here, we will describe the most and least frequently reported clonal complex, sequence types and ribotypes with their worldwide geographic distribution for the most important species involved in HAIs. (www.actabiomedica.it)

Key words: molecular epidemiology, healthcare associated infections

Background

Healthcare-associated infections (HAIs) are the most frequent and severe complication acquired in healthcare settings with high impact in terms of morbidity, mortality and costs. Many bacteria could be implicated in these infections, especially multidrug resistance bacteria (1), which had the capability to efficiently spread from patient to patient and to easily acquire antibiotic resistance determinants (2).

Microbial typing is often employed to determine the source of infections with an important role of bacterial epidemiological typing (3). In fact, although conventional microbial typing methods have been useful to describe the epidemiology of infectious disease, they are variable, poor reproducible and especially la-

bour intensive and time-consuming (4). So, many microbial typing technologies have been developed until to the the bacterial whole-genome sequencing (WGS) (5).

The choice of a molecular typing method therefore will depend on the skill level and resources of the laboratory and the aim and scale of the investigation. (6, 7)

The more classical molecular methods most often used and cited for HAIs are ribotyping, PFGE and MLST (2). In several studies the molecular investigation of pathogens involved in HAIs was performed with many microorganisms identified as causative agents such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Clostridium difficile*, *Acinetobacter baumannii*, *Enterobacter* spp., *Enterococcus* spp.,

Staphylococcus aureus and several more minor species.

The aim of this review is to describe the most and least frequently reported clonal complex, sequence types (STs) and ribotypes (RTs) with their worldwide geographic distribution for the most important species involved in HAIs.

Materials and methods

Search strategy and selection criteria

We carried out a systematic review to identify all study dealing with the identification of molecular epidemiology of HAIs of multi drug resistance bacteria (MDR).

We searched the main scientific databases (PubMed, Sciverse Scopus, Web of knowledge) for the following search terms: “molecular”; “epidemiology”; “healthcare”; “associated”; “infections”, using the function “AND”. The bibliographies of all relevant articles, including reviews, were screened for further references.

No language restrictions were imposed; papers in languages we were unable to read were translated using Google Translate. We developed the search terms in accordance with the Medical Subject Headings thesaurus, using a combination of test searches and via collaboration between independent researchers and knowledge users. After deleting duplicates, we further screened titles, abstracts, or entire articles using exclusion criteria. Screening was carried out independently by two authors (CG, SD). Any disagreement about eligibility between reviewers was resolved by a third author (RS and VLF). The first two authors extracted data from included papers using a data extraction form reviewed by the other co-authors (RS, VLF, AS, CA, GBC, FF). Every author contributes to the manuscript writing. These procedures comply with the PRISMA guidelines for reporting systematic reviews (8, 9).

Data extraction

Two independent reviewers (CG and SD) identified potentially relevant articles and collected the data.



Figure 1. Representation by order of the most and least reported STs and RTs for important species involved in HAIs

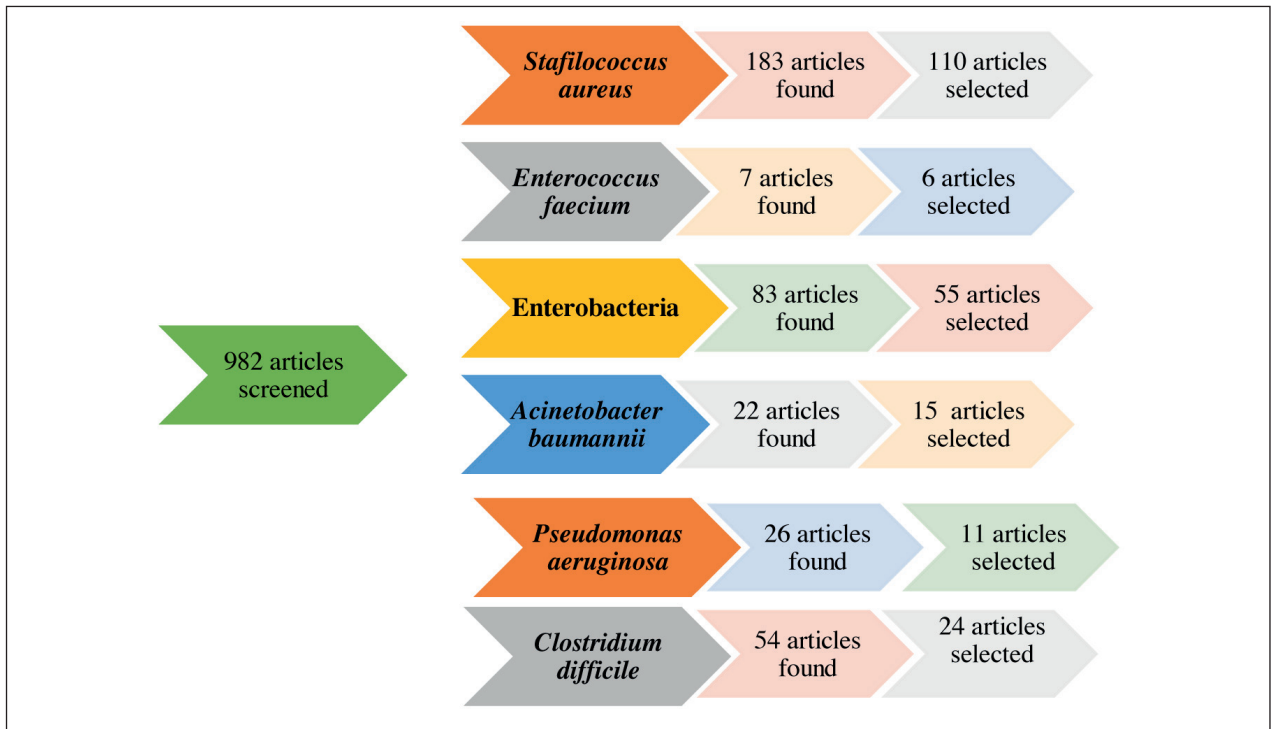


Figure 2. Flowchart of the evaluation and inclusion process for the review

Staphylococcus aureus

Pulsed-field gel electrophoresis (PFGE) is the “gold standard” for epidemiological investigation of methicillin-resistant *Staphylococcus aureus* (MRSA), but several DNA sequence-based methods have been developed (10). As regard the clonal distribution in North America the multilocus sequence type (ST) 8-IV (USA300 clone) predominated while community-associated MRSA in Europe is characterised by clonal heterogeneity. The most common European strain is the European clone (ST80-IV), despite reports of USA300 are also present in literature. Several MRSA clones have arisen in Europe, i.e. the ST398-V pig-associated MRSA clone in the Netherlands and Denmark. Here below, give to the highest number of articles detected we will provide a distribution of the main findings by country.

In Asia two pandemic HA-MRSA clones, ST 239 and ST5, are disseminated internationally, whereas the molecular epidemiology of CA-MRSA is characterized by clonal heterogeneity, similar to that in Europe

(11-19). Also, invasive *S. aureus* infections caused by PVL-positive strains are rare in Asia although several PVL-positive MRSA clones, predominantly ST8-SCCmecIVa and ST30-SCCmecIVc, were circulating and causing sporadic cases of invasive infections in the community and hospital settings (20).

In Shenzhen, China in a study at nine sentinel hospitals ST72-SCCmec type IV is the predominant clone confirming previously studies (21). Also, in India a study shows that healthcare-associated MRSA strains may harbour community-acquired MRSA genetic markers (22).

Another possible application of molecular methods was screening and identification of MRSA carriage, useful for controlling MRSA dissemination in hospitals, such as described by Wu TH et al in Taiwan. In this study the leading ST were ST59 (44.4%), ST45 (30.6%), and ST239 (8.3%) for all isolates (31) while in another study in South Korea instead of classic MRSA clones responsible for HAIs, ST 72 accounted for 52.8% of the isolates during 2013-2014 (21,23). Other sequenced type identified by Chen et

al were ST15 in CA and ST188 in HA (24,25). Despite ST239 was the predominant HA-MRSA clones in some studies was replaced by the continually growing ST5 and ST59, ST398, ST642 and ST107 (11, 26). The American strains was also be detected in some outbreaks (27). Finally some atypical clones could be detected (28).

In Europe each country had a distinct epidemiology, with ST8-IVc (USA500) being most prevalent, especially in France and Spain. The main clonal complex is CC8/239 and other prevalent ones were CC5 and CC22 (29-38).. Despite this some exceptions were described in literature; in a study conducted in UK WGS data from 20 historical outbreaks of MRSA were analysed and CC30 was the most common clonal complex followed by CC2 and CC8. CC30 was implicated in household infections while CC22 and CC8 in hospital settings, with the highest number of cases for CC8 (187 only for one outbreak) (38-40). Also, in Italy, similarly to other countries, prevalent strains were ST262 (34, 41-43) or the USA 300 one that in USA represented the dominant community- associated methicillin-resistant *S. aureus* lineage (44-52)). Although this, also USA100 clone, USA 300, USA500 and USA800 could be detected. Despite being relatively understudied, USA500 strains cause a significant burden of disease and were the third most common methicillin-resistant *S.aureus* (MRSA) strains identified in the U.S.A (52-55).

In Colombia, several HA-MRSA clones have been found, including the pediatric clone (CC5-ST5-SCC mec IV), the Brazilian clone (CC8-ST239-SCC mec III), and the Chilean/Cordobés clone (CC5-ST5-SCC mec I). Moreover, the CA-MRSA clone USA300 has been reported as causing hospital-acquired infections (56,57). ST 22 and ST 30 was identified in other studies (57-60). Also, ST 398 was detected in one sporadic case (61). While ST8-IV USA300 being the commonest clone in North America the ST30-IV Southwest Pacific clone established as the dominant clone in New Zealand for the past two decades, although recently unidentified PVL-negative ST5-IV spa t002 clone replaced it as the dominant CA-MRSA clone. Of particular concern was the finding of several successful and virulent MRSA clones from other geographic settings, including ST93-IV (Queensland

CA-MRSA), ST8-IV (USA300) and ST772-V (Bengal Bay MRSA) (61).

On December 2017, in Australia two prevalent HA MRSA clones were detected, ST22-IV and ST239-III while CA ones were characterised as ST93-IV, ST5-IV, ST1-IV and ST45-V_T. CA-MRSA, in particular the ST45-V_T clone has acquired multiple antimicrobial resistance determinants (62,63). These results confirmed previously study that highlighted the presence in invasive isolates predominantly of ST93 (26.6 %) and pvl positive (54.3 %) while non-invasive isolates were rarely ST93 (1.9 %) or pvl positive (7.4 %) (64).

More recently, methicillin-susceptible *S. aureus* (MSSA) belonging to CC398 have been increasingly reported as a cause of invasive infections in patients without livestock contact leading to bloodstream infections associated with high mortality (65).

In Africa BenDarif et al. isolated PVL-negative CC5 isolates most frequently (38%), more of which were similar to the HA USA100/800 strain type; CA-MRSA CC80 strains were the second most frequent (27%) followed by CC22. The minor groups (<10% each) were CC15, CC1, CC8/ST239, CC45, CC152, CC30 and CC88 (66, 67). In a recent study performed in South Africa 29.1% of cases were identified as MRSA infection (2.3% were considered CA-MRSA and 26.8% HA-MRSA). The most common sequence types were ST239 and ST612 of CC8 and a novel ST (ST4121) was obtained for one isolate (68). In a recent study HA MRSA (ST239 and ST22) and CA ones (ST80 and ST8) were found (69-70). In a public referral hospital in Kenya, in contrast to previous studies published, there was marked genetic diversity among clinical MRSA isolate and the predominant clonal complex was CC 5 (71,72). The European CA-MRSA clone in a study accounted for 14.1% of all HA MRSA infections (73). while the first description of the spread of the MRSA ST5-IVa clone was in 2014 (74). Finally, also USA 300 could be observed in some situations (75).

Other *Staphylococcus* spp

The gold standard for genotyping of *S. epidermidis* is pulsed-field gel electrophoresis (76), which

could also be utilized to characterize other species of *Staphylococcus*. Genotyping methods used in studies on the molecular epidemiology of CoNS are mainly based on two different techniques: DNA banding pattern analysis and DNA sequencing and recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry and Raman spectroscopy (77).

In a large study conducted in Iran the molecular epidemiology of *Staphylococcus epidermidis* and the comparison with a previously characterized collection of isolates origin from Northern Europe, Australia, and USA was performed. The study documented the dissemination of three MDRSE clones within and between hospitals in Iran and revealed an intercontinental spread of two clonal multidrug-resistant lineages (ST2 and ST5) in the hospital environment. Isolates of the predominant clones were significantly more frequently associated with multidrug-resistance and biofilm formation compared to nonclonal isolates. In particular three predominant PFGE clones were found. The PFGE patterns of the most common sequence type (PFGE type 040-ST2) showed 80% similarity to multidrug-resistant *S. epidermidis* (MDRSE) clinical isolates from eight hospitals in Northern Europe. The second most common (PFGE 024-ST22) showed a unique PFGE pattern, whereas the third most predominant genotype (PFGE 011-ST5) proved indistinguishable to the PFGE Co-ST5 identified in five hospitals in Northern Europe (78).

Methicillin-resistant *Staphylococcus lugdunensis* (MRSL) is increasingly recognized in healthcare and community settings. A study performed in China highlighted the diversified structures of SCCmec elements among MRSL strains and their close relationship with SCCmec elements harboured by CA-MRSA; the sequence type was in descending order ST3-SCCmec V, ST27-SCCmec V, ST3-SCCmec IV and ST42-SCCmec V with CC2 described such as dominant clonal complex in both community and hospital settings (79).

Enterococcus faecium

The main sequence type identified were ST78, ST17, ST18, ST192, ST16, ST117, ST203 and ST412 (2, 80-83).

Enterobacteriaceae

The prevalence of carbapenemase-producing *Enterobacteriaceae* (CPE) is increasing worldwide. Regarding the resistance determinants, SHV, TEM, OXA-1-like and CTX-M-gp1 were predominant enzymatic variants, whereas CTX-M-gp9, CTX-M-gp2, KPC, VIM, GES, OXA-48-like, NDM and OXA-23-like were considered emerging enzymes (84). Also, some association between several risk factors and ESBL-KP infection such as length of hospitalization, use of cephalosporins, use of quinolones, presence of a nasogastric tube, presence of an intravenous catheter, mechanical ventilation and cerebrospinal fluid drainage were found (85-126).

Escherichia coli

Extra-intestinal pathogenic *Escherichia coli* are a significant cause of urinary tract infection and bacteraemia. Many studies were performed to investigate the prevalence and molecular epidemiology of ESBL-producing *Escherichia coli* causing HCAs and CA infections. The prevalent clones were ST 131, ST 38, ST 648, ST 4120, ST 95, ST 69, ST10 and ST 473 (85-99). Also, such as the other bacteria some other clones could be described. In a study conducted in UK Trends in ExPEC serogroups were investigated: serogroups O25, O6, and O2 dominated and they were linked to the major ExPEC STs as follows: ST131-O25, ST73-O6, ST127-O6, and ST95-O2 (87), confirming other studies (100-101). In a Manchester hospital a large *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Escherichia coli* outbreak was investigated: genomic analysis identified the spread of ST216 among patients and in the environment. Patient relocation and plumbing replacement were associated with control of outbreak; however, environmental contamination with CRE and patient CRE acquisitions recurred rapidly following this intervention (102).

Klebsiella pneumoniae

Klebsiella pneumoniae is an opportunistic pathogen and leading cause of HAIs infections. The main clones described were ST258, ST101, ST147, ST 48,

ST 512, ST 15 and ST 14 (99,103-107). Some exceptions could be observed. In a multicentre prospective cohort study in Spain *K. pneumoniae* ST405 predominated (108,109). On November 2015, a KPC-producing *Enterobacteriaceae* outbreak occurred in a general hospital in South Korea due to a clonal spread of *K. pneumoniae* ST307 carrying a self-transferable IncX3-type plasmid harboring bla_{KPC-2}. Sporadic emergence of *K. pneumoniae* ST697 and a ST11 isolate were observed (110).

K. pneumoniae were carbapenemase producers, especially OXA-48-like (111-112). In an Italian study carbapenemase producers belonged to 10 different STs, with ST175 and ST621 being the most common lineages (113). The spread of carbapenemase-producing *Enterobacteriaceae* (CPE) is a great problem also in Russia, where in a study most of isolated strains under study were multi drug resistant; MDR mechanisms were based on carrying of epidemic extended-spectrum beta-lactamase bla CTX-M-15 gene, carbapenemase bla OXA-48-like gene and class 1 or 2 integrons (114). In a study performed in Colombia 85.7 % of *K. pneumoniae* were positive for KPC carbapenemase, especially KPC-2 and 3 KPC-3, while for *P. aeruginosa* and *E. cloacae*, most isolates were non-carbapenemase producing (87.5 %). Molecular analysis revealed that most isolates belonged to ST14 for KP while ST170 and ST1804 were found in *P. aeruginosa* (108).

Apisarnthanarak et al detected that among 71 patients with HA infection due to an ESBL-producing strain of *E. coli* or *K. pneumoniae*, the gene for CTX-M, with or without other ESBL genes, was identified in all patients infected with an *E. coli* strain and in 90% patients infected with a *K. pneumoniae* one (107). In another study performed in Brunei ST 231 was the most isolated type, which may be representatives of a high-risk CRKP clone disseminating across Southeast Asia. Resistance of isolated strains was due to the production of OXA-232 and CTX-M-15 β -lactamases (109). Other clones could be associated with outbreak such as ST 307 or ST697 (115). In France, a study investigates epidemiological links between apparently unrelated cases of OXA-48-producing *Klebsiella pneumoniae* (Kp OXA-48) colonisation or infection. This study showed that environmental reservoirs should be considered as a source of CPE transmission (112),

such as confirmed in other studies, i.e. in a Tunisian Hospital (ST167 and ST131) showing that strict control measures should be established to minimize this problem (113-116). *K. pneumoniae* could produce several carbapenemase: i.e. New Delhi metallo- β -lactamase-1 (NDM-1) is among the most recently discovered carbapenemases (117). In the last decade, hospital outbreaks involving KPC-producing *K. pneumoniae* have been predominantly attributed to isolates belonging to clonal group (CG) 258. However, results of recent epidemiological analysis indicate that ST 307, is emerging in different parts of the world and is a candidate to become a prevalent high-risk clone in the near future. A study showed that the ST307 genome encodes genetic features that may provide an advantage in adaptation to the hospital environment and the human host, in fact compared with the ST258 clone, capsulated ST307 isolates showed higher resistance to complement-mediated killing (118). In a study 31 patients were examined after returning to Poland from a trip to South and South-East Asia. The presence of New Delhi Metallo- β -lactamase-1 producing *Escherichia coli* and *Klebsiella pneumoniae* was confirmed in three patients (9.7%) returning to Poland from travels to India. All the positive patients were hospitalized during the trip in a New Delhi hospital (117).

Other *Enterobacter* spp and miscellaneous

In UK a new *E. cloacae* complex isolate belonged to a novel ST (ST829) highlighting the importance of phenotypic tests to detect carbapenemase activity when molecular assays are negative for the 'big 5' carbapenemase families to understand the possible circulation of rarer carbapenemases in clinical settings (119)

In a Spanish study a high number of OXA48KP isolates showed multidrug resistance (ST 15 and ST 29) and was associated with a high mortality and mainly hospital-acquired (120,121).

A hospital-wide point prevalence study and active surveillance were performed by Forde C. et al. to assess the prevalence of CRKP infection/colonization. During the surveillance period *K. pneumoniae* was the most frequently occurring species, followed by *Enterobacter* spp. All isolates involved in both outbreaks harboured the blaKPC gene, as demonstrated by PCR (122).

A multicenter study showed that in pediatric patients the ST 131 was the most prevalent sequence type among all resistant *E. coli* isolates (30%), and the CG 258 was the most prevalent allele among all resistant *K. pneumoniae* isolates (10%) (123).

Carbapenemase producing *Citrobacter freundii* infections are still uncommon in European countries, In a study were identified ST11, ST18, ST22 and ST64 and 6 new STs (ST89, ST90, ST91, ST92, ST92 and ST94). In this study of Villa J et al the dissemination of the bla_{VIM-1} gene among various clones suggests a successful horizontal transfer of integron carrying elements that play a dominant role in the development of multidrug resistance in *Enterobacteriaceae* (124).

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a leading cause of HAIs and often shows MDR phenotypes. It could be related also to a wide variety of clinical syndromes in neonatal intensive care unit patients, including sepsis, pneumonia, meningitis, diarrhea, conjunctivitis and skin infections. In these cases, molecular investigation and WGS provided detailed information without the need for further typing and could be useful to understand outbreak situations rapidly and with certainty (125-126). The most frequent isolates belong to ST235, ST111, ST175, ST 233, ST 244, ST 395, ST 277 and ST 274 (2, 125-126).

Also, Carbapenem-resistant isolates of *Pseudomonas aeruginosa* producing metallo- β -lactamases (MBLs) are increasingly reported worldwide and often belong to particular 'high-risk clones'. In particular multidrug-resistant *Pseudomonas aeruginosa* expressing VIM-metallo-beta-lactamase is an emerging infection control problem. The source of many such infections is unclear, though there are reports of hospital outbreaks of *P. aeruginosa* related to environmental contamination, including tap water (127). In an Italian study overall, 5.1% isolates were positive for carbapenemase genes, including bla_{VIM}, bla_{IMP} and bla_{GES-5}, while the remaining ceftolozane/tazobactam-resistant isolates tested negative for carbapenemase production. In a study, an outbreak in an intensive care burn unit was due especially to DLST 1-18; carbapenemase producers belonged to 10 different STs, with ST175

and ST621 being the most common lineages (128). In a UK study VIM-type MBLs predominated (91% of all MBLs found), but a few IMP- and NDM-type enzymes were also identified. Diverse VNTR types were seen, but 86% of isolates belonged to six major complexes. MLST of representative isolates from each complex showed that they corresponded to ST 111, 233, 235, 357, 654 and 773, respectively (129,130). Despite in several studies most isolates carried VIM-2, others carried IMP-1 or IMP-13, NDM-1, VIM-2 and IMP-18 or no metallo-beta-lactamase (MBL) gene were identified (194-195). In an Estonian study clinically relevant beta-lactamases (OXA-101, OXA-2 and GES-5) were found in 12% of strains, 27% of which were located in plasmids; whereas ST108 was associated with localized spread in one hospital and mostly carbapenem-resistant phenotype, ST260 strains occurred in all hospitals, mostly with multi-resistant phenotype and carried different resistance genotype/machinery (131).

Acinetobacter baumannii

Acinetobacter baumannii is an important hospital-acquired pathogen in healthcare facilities that frequently causes bacteraemia and ventilator-associated pneumonia in intensive care units. *Acinetobacter baumannii* can be isolated from various sites in the hospital environment like medical equipment, bed linen, medical personnel and indwelling catheters. Multidrug resistance in the nosocomial pathogen *Acinetobacter baumannii* limits therapeutic options and impacts on clinical care. Resistance against carbapenems, a group of last-resort antimicrobials for treating multidrug-resistant (MDR) *A. baumannii* infections, is associated with the expression (and over-expression) of carbapenemases encoded by the bla_{OXA} genes. The most common species isolated were ST 208, ST 92, ST 195, ST 75, ST 231, ST 191, ST 218 and ST 236 (132,133).

In a study performed in three hospitals in southern Vietnam from 2012 to 2014 antimicrobial resistance was common (74% of isolates were both MDR and XDR). High-level imipenem resistance for 91.6% of the XDR imipenem-nonsusceptible organisms, of which the majority (86.7%) harboured the bla_{OXA-51}

and blaOXA-23 genes associated with an ISAbal element (134).

The first description of NDM in *A. baumannii* in newborn units in Turkey was found in 2016 (135). In a study performed in South Africa all the *A. baumannii* isolates showed a high MDR (100 % resistance to ampicillin, amoxicillin, cefuroxime, cefuroxime axetil, ceftazidime, ceftazidime, cefepime, imipenem, meropenem, gentamicin, ciprofloxacin and trimethoprim/sulfamethoxazole). The most dominant ST among the collected isolates was ST758, member of the EUI group, but other ST identified were ST258, ST339, ST502 and ST848. The M-PCR assays showed that 99 % of the isolates contained the OXA-51 gene and 77 % contained the OXA-23 gene and was not restricted to a specific ST (136).

In a study in Brazil 91.9% isolates were resistant to imipenem and 98.8% were susceptible to colistin. The blaOXA-23 gene (78.2%) and its upstream insertion ISAbal (55.2%) were predominant, followed by blaOXA-24 (55.2%) and blaOXA-143 (28.7%). The blaOXA-23 gene and ISAbal were independently associated with resistance to imipenem ($P < 0.05$). Different sequence types (STs) were detected among the 35 isolates: ST1 (25.7%), ST162 (22.8%) and ST730 (17.1%) were the most common, and four new STs were identified. The isolates were grouped into five clonal complexes (CC1, CC15, CC79, CC108 and CC162) (137). In a study conducted in South America the phenotypic identification of isolated showed that the isolates belong mainly to *A. calcoaceticus*-*A. baumannii* complex. All of them were multi-resistant to almost the whole antibiotics except to tigecycline and sulperazon, and they were grouped into five (I to V) different antibiotypes, being the antibiotype I the most common (50.0%). The percent of beta-lactamases detected was: blaTEM (17.3%), blaCTX-M (9.6%), blaVIM (21.2%), blaIMP (7.7%), blaOXA-58 (21.2%), and blaOXA-51 (21.2%). The phylogenetic tree analysis showed that the isolates were clustering to *A. baumannii* (74.1%), *A. nosocomialis* (11.1%) and *A. calcoaceticus* (7.4 %). Besides, the integron class 1 and class 2 were detected in 23.1% and 17.3% respectively (138).

In a study performed in Brazil isolates were only susceptible to amikacin, gentamicin, tigecycline, and

colistin, and contained the ISAbal insertion sequence upstream of blaOXA-23 and blaOXA-51 genes. Twenty-six OXA-23-producing *A. baumannii* strains belonged to the ST79 (CC79) clonal group, and patients infected or colonised by these isolates had a higher mortality rate (34.6%) (139).

In some studies, high mortality rate was detected for infections with some ST such as ST457 a clone that exclusively shared a few virulence factors with the hypervirulence strain LAC-4, including a capsule biosynthesis locus (KL49) that is supposed to be important for the hypervirulence in LAC-4 (140). Carbapenem resistance in *Acinetobacter baumannii* in China was mainly mediated by OXA-23-like carbapenemases, while OXA-24/40-like carbapenemases were rarely identified with OXA-72 as one variant of this latter (140). Also, in other countries the same carbapenemase was identified such as i.e. in Iran: in a study almost all *A. baumannii* isolates were extensively drug-resistant (98%) and carried blaOXA-23-like (98%) and class 1 integrons (48%). PFGE and MLST analysis identified three major genotypes, all belonging to clonal complex 92 (CC92): ST848, ST451 and ST195. CC92 has previously been documented in the hospital setting in northern Iran, and ST195 has been reported in Arab States of the Persian Gulf (141). In African study ST391 was the predominant ST detected, 70.5% of which harbored blaOXA-23 alone, both blaOXA-23 and blaKPC in 11.8%. Carbapenem resistance due to blaOXA-23 carbapenemase was detected in 72% of isolated, followed by blaOXA-23 concomitant with blaKPC in 14%, while blaNDM with blaOXA-58 in 6% and blaNDM alone in 1 case (2%) (142). Other sequence types were identified such as ST1, ST162 and ST730 and ST 22 and ST26 in Hong Kong (140).

Clostridium

Clostridium difficile is an emerging cause of health-care-associated infections with increasing frequency and severity attributed to highly virulent ribotypes such as 027. The changing epidemiology of *Clostridium* and the emergence of epidemic 027 necessitate continued surveillance to identify shifts in antibiotic susceptibility (143-145). *Clostridium difficile* can be charac-

terized according to its ribotyping which is performed using the polymerase chain reaction. Several different ribotypes have been associated with *Clostridium difficile* infection (CDI). The ribotypes 001, 002, 014, 046, 078, 126, and 140 have been found to be prevalent in the Middle East. In Asia, ribotypes 001, 002, 014, 017, and 018 are more prevalent (2, 143-164).

Predominant strains	Country
RT 001, 014, 020, 027, 078a	Europe and North America
001, 002, 014, 046, 078, 126, 140	Middle East
002, 014, 017, 018	Asia

Ribotype 027 was found to have reduced susceptibility to metronidazole, rifampicin, moxifloxacin, clindamycin, imipenem, and chloramphenicol and also it leads to severe disease presentation, high morbidity and mortality rates, spread more easily within the hospital because they can resist the hospital environment, cleaning, and disinfectants (215-217). In fact, the cost of CDI is estimated to be about 3000 million euro/year in Europe and is expected to almost double over the next four decades (165). ClosER, currently the largest pan-European epidemiological study of *C. difficile* ribotype distribution and antibiotic susceptibility, aimed to undertake antimicrobial resistance surveillance pre- and post-introduction of fidaxomicin. In this study ribotypes 027, 014, 001, 078, 020, 002, 126, 015 and 005 were most frequently isolated, and emergent ribotypes 198 and 356 were identified in Hungary and Italy, respectively. All isolates were susceptible to fidaxomicin, with scarce resistance to metronidazole (0.2%, 6/2694), vancomycin (0.1%, 2/2694) and tigecycline (0%). Rifampicin, moxifloxacin and clindamycin resistance was evident in multiple ribotypes. Epidemic ribotypes (027/001) were associated with multiple antimicrobial resistance, and ribotypes 017, 018 and 356 with high-level resistance (146,149,150). This data was confirmed in other study both in Italy that in other countries (148-160). Also, in Portugal RT027 was the most frequent among healthcare facility-associated isolates (19.6%), while RT014 was the most common among community-associated isolates (12%) (158). In Asia the toxigenic ribotypes 043 and 017 were most common (both 14%) (159) and

the latter was also found in a study in Germany (160). Although approximately 30-40% of children <1 year of age are *Clostridium difficile* colonized; they could represent a reservoir for adult CDI. In New Zealand PCR ribotyping was performed on 32 *C. difficile* isolates cultured from the stool specimens of children with CDI founding that most belong to ribotype 014 (161). Similar findings were discovered in a Croatian University Hospital where except to the ribotype 014 the 001 was the most prevalent one (162). In Australia were found RTs 014/020, 002, 056 and 070, similar to a previously study conducted in 2010. Proportions of RTs 014/020 and 002 remained similar respect to the past, while RTs 056, 015, 017 and 244 increased in prevalence (163). Also, other clones could be isolated in healthcare settings such as ribotype sa026 and 176 (164).

Conclusion

Undetected pathogen clusters can often be a source of spreading in-hospital infections. Unfortunately, detection of clusters can be problematic because epidemiological connection is not always easily established. Infection prevention and control (IPC) measures, however, are most effective when applied at the earliest possible stage.

Implementing daily routine molecular typing is effective for detecting and analyzing pathogen clusters. Falsely suspected outbreaks can be quickly resolved, whereas actual outbreaks can be identified faster, so that targeted IPC measures can be applied earlier; also the molecular epidemiology is important to identify if the causative microorganism of the infection is really of environmental origin, in this case carrying out ad hoc sanitization procedures (165,166).

In fact, persistent contamination of hospital surfaces contributes to HAI transmission, and it is not always efficiently controlled by conventional cleaning, which does not prevent recontamination, has a high environmental impact and can favour selection of MDR strains (166-175).

Molecular epidemiology is an indispensable tool and should be part of a multidisciplinary approach in the proper management of HAI. In fact, although

these bacteria could lead to infections especially in immunodeficient patients sometimes is possible to found case in immunocompetent ones, leading to high economic cost for the healthcare national system and also to unexpected death (176-179).

So, it is important to apply the best practices (such as vaccination, sanification, apply and improvement of guidelines, etc...) in time to reduce the prevalence of microorganism into healthcare area. In like of this scenario also the immunization of HCWs had a big role to prevent infection both of patients that of their colleagues, despite the spread of vaccine hesitance (180-193).

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