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Original Article

Molecular epidemiology of meticillin-resistant *Staphylococcus aureus* in Italian cystic fibrosis patients: A national overview

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Abstract

Background: The genetic background, transmissibility and virulence of MRSA have been poorly investigated in the cystic fibrosis (CF) population. The aim of this multicentre study was to analyse MRSA strains isolated from CF patients attending nine Italian CF care centres during a two-year period (2004–2005). All CF patients infected by MRSA were included.
Method: Antibiotic susceptibility testing, SCC*mec* typing, Panton–Valentine Leukocidin (PVL) production, and Multi Locus Sequence Typing (MLST) analysis were carried out on collected isolates (one strain per patient).
Results: One hundred and seventy-eight strains isolated from 2360 patients attending the participating centres were analysed. We detected 56 (31.4%) SCC*mec* IV PVL-negative strains, with a resistance rate of 80.3% to clindamycin and of 14.5% to trimethoprim/sulphamethoxazole. MLST analysis showed that many isolates belonged to known epidemic lineages. The largest clone grouping of 29 isolates from 6 centres had the genetic background (ST8-MRSA-IV) of the American lineages USA300 and USA500, thus demonstrating the diffusion of these strains in a population considered at risk for hospital associated infections.
Conclusions: Known MRSA epidemic clones such as USA600, USA800, USA1100, and UK EMRSA-3 were described for the first time in Italy. The diffusion of MRSA strains with high pathogenic potential in the CF population suggests that analysis of the MRSA strains involved in pulmonary infections of these patients is needed.

Keywords: MRSA; SCC*mec*; MLST; Cystic fibrosis

1. Introduction

Cystic fibrosis (CF) patients' lungs are the ideal habitat for several bacterial species and *Staphylococcus aureus* is usually the first pathogen detected in the respiratory tract. In the pre-antibiotic era, this pathogen was the major cause of morbidity and mortality. Recently a further cause for concern has been the emergence of meticillin-resistant *Staphylococcus aureus* (MRSA) [1].

Data from the American CF Foundation Patient Registry shows that the prevalence of MRSA infection in CF has increased from 6.1% in 2001 to 23.7% in 2009 [1].

Although the contribution of MRSA infection to lung damage in CF patients is incompletely understood, some studies indicate that the presence of MRSA in CF patients' airways may worsen their clinical condition [2]. It has also been recently reported in a large CF population that persistent MRSA infection affects lung

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function and survival [3,4]. Although guidelines of the European Society of Cystic Fibrosis Society suggest attempting to eradicate MRSA, therapeutic strategies still vary among CF centres [5].

MRSA was first recognised as being acquired from hospitalised patients (HA-MRSA), but the onset of MRSA infection outside the hospital setting, due to community-acquired strains (CA-MRSA), has recently been described with increasing frequency [6]. Although HA-MRSA strains are known to be responsible for infections in hospitalised patients, highly virulent CA-MRSA strains are increasing worldwide and are implicated in serious infections, including necrotising pneumonia and severe sepsis, as well as fatal outbreaks [7]. Recently CA-MRSA strains have been found in hospitals, displacing classic hospital-associated strains, consistent with the hypothesis that the former may be more virulent [8].

CA-MRSA and HA-MRSA can be distinguished on the basis of a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*) integrated into the MRSA chromosome. Currently, eight SCC*mec* types are recognisable: I, II, III, IV, V, VI, VII and VIII [9–12].

HA-MRSA are characterised by SCC*mec* types I, II, III and VIII while SCC*mec* types IV, V, VI and VII are mainly associated with CA-MRSA isolates. In SCC*mec* type I, no antibiotic resistance determinants except for those carried by *mecA* (beta-lactams) are found. In contrast, types II and III contain multiple determinants for non-beta-lactam antibiotic resistance and provide a molecular explanation for the multiple drug resistance often documented in MRSA isolates circulating in healthcare environments.

CA-MRSA strains are more virulent than HA-MRSA strains due to the production of many virulent factors such as Panton–Valentine Leukocidin (PVL), which can be associated with necrotising pneumonia [13].

To date, few studies have investigated the genetic background, transmissibility, antibiotic susceptibility and virulence of MRSA strains in the CF population [14–19].

The prevalence of CA-MRSA is increasing in CF patients with newly acquired strains being mostly represented by CA-MRSA indicating that CA-MRSA strains are now circulating in the CF population and are responsible for new colonisations [20,21].

The goal of this study was to investigate the epidemiology and genetic background of MRSA infecting the CF population. This knowledge could help identify MRSA with different pathogenicities and consequently improve prevention and treatment strategies.

2. Materials and methods

2.1. Patients

A two-year (2004–2005) multicentre study on MRSA involved 2362 CF patients attending 9 (40.9%) out of 22 Italian Cystic Fibrosis (CF) centres. All CF patients infected by MRSA in the studied period were included. No CF patients were shared among different CF centres. Patients were considered infected in the case of one positive culture. One MRSA strain isolated from respiratory samples of each of the MRSA infected patient was collected and then stored at −80 °C.

2.2. Bacterial strains

All collected bacterial isolates were identified as *Staphylococcus aureus* by typical colony morphology on selective culture media (Mannitol Salt 2 Agar, Biomerieux) and positive results of the Slidex Staph Plus (Biomerieux). MRSA isolates were identified using Oxa Screen Test Agar (Becton and Dickinson) and MRSA ID (Biomerieux).

2.3. Characterisation of SCCmec types

SCC*mec* typing of collected isolates was performed as previously described [9,22,23]. MRSA isolates were tested for the presence of the genes (*lukS-lukF*) encoding a subunit of PVL, following the protocol of Lina and colleagues [24].

2.4. Evaluation of antimicrobial susceptibility

Antibiotic susceptibility was evaluated by assessing the activity of a large panel of drugs. The antibiotic susceptibility profile of MRSA strains collected was evaluated by disc diffusion test on Mueller–Hinton agar. Susceptibility to glycopeptides was evaluated by Etest macromethod as previously described [25].

For isolates that tested resistant to erythromycin but susceptible to clindamycin by single-agent testing, a D-zone test to detect inducible clindamycin resistance was performed.

All assays were performed according to the Clinical Laboratory Standards Institute Guidelines [26].

Susceptibility patterns of MRSA isolates with different characteristics were compared. Proportions were analysed using chi-square and Fisher's exact test to assess for significance ($p<0.05$).

2.5. Multilocus sequence typing (MLST) analysis

MLST was performed on MRSA isolates for epidemiological purposes as previously described [27].

3. Results

One hundred and seventy-eight (7.5%) putative MRSA strains were collected from as many patients out of 2362 CF patients attending CF centres in Italy in the period (2004–2005). All bacterial isolates were then identified as MRSA.

3.1. Identification of SCCmec type

The SCC*mec* type of 153 of 178 (85.9%) MRSA strains was identified. We recognised 88 (49.5%) strains with SCC*mec*I, 56 (31.4%) strains with SCC*mec*IV, 2 (1.2%) with SCC*mec*II and 7 (3.9%) with SCC*mec*III. Twelve strains (6.7%) showed a multiple SCC*mec* profile and 13 (7.3%) strains had an undetermined SCC*mec* type.

All MRSA isolates were tested for the presence of the gene *lukS-lukF* encoding for PVL, but no positive strains were found. HA-MRSA strains were mainly SCC*mec* type I.

3.2. Antimicrobial susceptibility testing

The resistance rates of the SCC*mec*I and SCC*mec*IV strains are compared in Table 1. No resistance against glycopeptides (vancomycin and teicoplanin) and oxazolidinones (linezolid) was found, the most active antibiotics among the other classes were tetracyclines (minocycline and doxycycline with 4.4% and 13.4% of resistant strains respectively), trimethoprim/sulfamethoxazole (13.2%), and rifampin (33%). SCC*mec*I demonstrated a higher resistance rate profile in comparison to SCC*mec*IV strains for gentamicin and doxycycline. The difference in gentamicin resistance between the two groups was statistically significant ($p<0.05$).

3.3. MLST analysis

MLST analysis showed that 98 isolates belonged to known epidemic lineages (Table 2). The largest clone was ST8-MRSA-IV which accounted for 29 strains collected from 6 different centres. The second most represented epidemic clone (26 strains from 6 CF centres) was UK/EMRSA-3 (ST5-MRSA-I), followed by the Southern Germany clone (ST228-MRSA-I) grouping 20 isolates from 6 centres, the Pediatric/USA800 (ST5-MRSA-IV) I and the Iberian clone (ST247-MRSA-I), both grouping 9 strains isolated from 2 to 7 CF centres respectively.

Other important MRSA lineages such as USA100 (ST5-MRSA-II), the Brazilian/Hungarian clone (ST239-MRSAIII), the Southwest Pacific, Berlin/USA600 clone (ST30-MRSA-IV and ST45-MRSA-IV respectively) were represented by few isolates (1 to 2 strains).

Table 1
Characteristics and antibiotic resistance profiles of MRSA strains.

Antimicrobial agent	Prevalence of resistant strains n (%)			
	MRSA (total) (n=138)	SCC*mec*I (n=82)	SCCmecIV (n=56)	p
Tobramycin	86.9	83.6	90.7	NS
Gentamicin	75.3	83.7	62.9	<0.05
Ciprofloxacin	75	74	78.1	NS
Levofloxacin	55.5	58.7	50.9	NS
Rifampin	33	30.8	36.3	NS
Minocycline	4.4	5.1	3.5	NS
Doxycycline	13.4	17.9	7.1	NS
Erythromycin	90.5	95	83.9	NS
Clindamycin [a]	80.4	82.5	80.3	NS
Trimeth./sulfam	13.2	12.3	14.5	NS
Linezolid	0	0	0	–
Vancomycin	0	0	0	–
Teicoplanin	0	0	0	–

[a] Includes inducible resistance.

Table 2
MLST analysis of MRSA strains.

Sequence type	SCC*mec* type	No. of strains (centres)	Clone
5	I	26 (a,b,c,f,g,h)	UK/EMRSA-3
5	II	1 (h)	USA100
5	IV	9 (a,h)	Pediatric/USA800
8	IV	29 (a,b,d,e,f,h)	USA500 and USA300
30	IV	2 (h)	Southwest Pacific
45	IV	1 (c)	Berlin/USA600
228	I	20 (a,b,c,e,g,h)	Southern Germany
239	III	1 (g)	Brazilian/Hungarian
247	I	9 (a,b,c,f,g,h,i)	Iberic
Total		98	

4. Discussion

Few studies have been published about the genetic background of MRSA in CF patients. The increasing prevalence of CA-MRSA in the CF population has been demonstrated, as well as the fact that they are primarily responsible for new colonisations in these patients. PVL-positive CA-MRSA strains have already been isolated from CF patients and their diffusion demonstrated [20,28].

In this study a high prevalence (31.4%) of SCC*mec*IV, commonly associated with CA-MRSA was found [14]. All these SCC*mec*IV isolates were negative for PVL genes, frequently described in CA-MRSA.

In contrast with other reports [20], only 1.1% of the HA-MRSA isolates harboured SCC*mec* II while the most represented was SCC*mec*I (49.4%). This latter evidence concords with a recent paper demonstrating a high prevalence of ST228-MRSA-I clone in CF patients [15].

Regarding antibiotic susceptibility patterns, previous findings have demonstrated that most CA-MRSA isolates are more susceptible than HA-MRSA to tetracycline–minocycline, clindamycin, gentamicin, rifampicin and trimethoprim/sulfamethoxazole [29]. SCC*mec* type IV strains were more susceptible than SCC*mec*I to gentamicin and doxycycline, with the difference in gentamicin resistance rates between the two groups being statistically significant ($p<0.05$).

Clindamycin resistance was high both for SCC*mec*IV and SCC*mec*I strains (80.3% and 82.5% respectively). Most CA-MRSA are associated with good susceptibility to clindamycin [30], but high clindamycin resistance has been demonstrated in CA-MRSA isolated from different countries in outpatients and nosocomial settings including patients with CF [31,32]. Unlike other studies a good susceptibility rate to clindamycin is not associated with SCC*mec*IV [33].

We found that the epidemiological picture of MRSA in our study was different from the situation in the United States where only a few lineages predominate such as USA500 and PVL-positive USA300 [34]. The frequency of isolation of USA300 has recently increased worldwide, being reported in Germany, Belgium, Denmark, the Netherlands, the UK, France, Austria, Spain and Japan [35–37]. So far few reports describe isolated infections by USA300 in nosocomial and outpatient settings in Italy [38,39].

The epidemiological picture of MRSA delineated by MLST analysis in this study showed that most of the SCC*mec*IV isolates (73%) belonged to known epidemic lineages with global diffusion.

The most represented clone identified by MLST analysis was ST8-MRSA-IV, grouping 29 isolates in 6 different Italian CF centres. This is the genetic background characteristic of the American lineages USA500 and USA300 [40]. The other most commonly found clone, characterised by SCC*mec*IV, among the Italian isolates was the Pediatrics/USA800 with 9 strains isolated from two centres.

The HA-MRSA isolates represented the majority of MRSA strains isolated from our study (54.4%). Fifty-seven (58.7%) out of 97 HA-MRSA strains belonged to epidemic lineages, the most frequent being ST5-MRSA-I described in the UK with 26 isolates collected from 6 different centres, followed by the Southern Germany (ST228-MRSA-I) clone with 20 strains from 6 centres. The Iberian clone, an HA-MRSA lineage frequently associated with hospital outbreaks in European Countries [40], was represented by 9 strains isolated from 7 centres.

The epidemiological picture of MRSA emerging from this national overview showed that 98 (55%) out of 178 isolates belonged to important epidemic lineages involved in serious outbreaks worldwide. This was the first description in Europe of a large spread of MRSA strains with the genetic background of important American epidemic clones. This was also the first description in Italy of the following worldwide diffused epidemic strains: Berlin (USA600), Pediatric (USA800), Southwest Pacific (USA1100), and UK EMRSA-3 [40]. One limitation of this study was that analyses were performed on strains isolated in 2004 and 2005, therefore this first overview epidemiology on MRSA patients in Italy needs to be updated by studying strains of more recent isolates [41].

The high prevalence of MRSA isolates representing epidemic clones, characterised by severe virulence and transmissibility, suggests that the epidemiology of MRSA is changing rapidly. The diffusion in the CF population of MRSA strains characterised by a high pathogenic potential could have a severe impact on the clinical status of CF patients, and therefore careful surveillance and close monitoring of MRSA infections is essential.

Conflict of interest

Dr G. Taccetti is serving as an advisor to Chiesi Farmaceutici. The other authors declare no competing interests.

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