Foggo-Osseyran et al., J Medical Microbiol Diagnosis 2015, S:5
http://dx.doi.org/10.4172/2161-0703.S5-006

Medical Microbiology & Diagnosis

Research Article Open Access

Molecular Typing of Methicillin-Resistant *Staphylococcus aureus* in Bermuda

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Abstract

It has been well established that inappropriate antimicrobial use has contributed to the emergence and spread of multi- drug resistant bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA). This is the first report of molecular typing of MRSA isolates from Bermuda. Sixty two MRSA isolates obtained from patients in a community hospital and out-patients from the community were subjected to molecular typing using pulsed-field gel electrophoresis (PFGE). The majority of the MRSA isolates belong to the USA 100 clonal type (43%). A clonal group similar to USA 300 was isolated from out-patients (29%) compared to in-patients (9%). In addition, retrospective analysis of susceptibility testing revealed that both the USA 100 and USA 300 clonal types were fully susceptible to vancomycin, gentamicin and linezolid. Furthermore, greater than 90% of both clonal types were susceptible to trimethroprim-sulfamethoxazole and tetracycline. Empirical therapy using agents active against MRSA should be considered for patients presenting with skin and soft tissue infections.

Keywords: Molecular typing; Healthcare-acquired MRSA; Community-acquired MRSA

Introduction

Although initially recognized as an important nosocomial or healthcare associated pathogen, MRSA is now endemic in the community [1]. Risk factors for healthcare-associated MRSA (HA-MRSA) have been well defined and include hospitalization, surgery, and residence in a long-term care facility, and use of indwelling catheters or other percutaneous medical devices [2]. Community-associated MRSA (CA-MRSA) infections are known to be caused by virulent strains which can affect healthy individuals who had none of the previously mentioned HA-MRSA risk factors [3]. Furthermore, CA-MRSA infections most commonly manifest as skin and soft tissue infections, but more invasive infections, including sepsis and necrotizing pneumonia, also occur [4]. However, it is important to note that CA-MRSA strains have been increasingly reported as an important cause of nosocomial infections, indicating that they may become endemic in hospital settings [5]. It has been well established that MRSA is an increasing problem in the USA, Europe, Asia, South America and the Caribbean. In this study, we report the first molecular typing results of MRSA isolates for Bermuda.

Materials and Methods

In this study, we carried out molecular typing on 62 MRSA isolates which were obtained from in-patients and out-patients from the community presenting with skin and soft tissue infections. We considered an infection to be of healthcare onset if the MRSA culture was obtained >72 hours after a patient was admitted to the hospital and the patient had no evidence of infection at the time of admission. A MRSA culture obtained within 72 hours of presenting to the Emergency Department of the hospital or a physician in the community with evidence of infection was considered an indication of community-associated infection. Isolates were previously tested for oxacillin resistance by disk diffusion and susceptibility testing was carried out using the Vitek II automated system (BioMerieux,Inc,Durham,NC).

The MRSA isolates were genotyped by pulsed-field gel electrophoresis (PFGE) using a protocol developed in the Division of Medical Microbiology at the Johns Hopkins Hospital, USA. Briefly, overnight broth cultures of the organisms were pelleted; bacterial DNA was extracted in agarose plugs using a solution containing lysostaphin and lysozyme. Restriction enzyme digestion was performed using *Sma* 1. Restriction endonuclease fragments were analyzed by PFGE using a contour-clamped homogenous electric field DR-11 apparatus (BioRad Laboratories, Inc, Hercules, CA) set at 14°C; initial switch, 5 seconds, final switch 50 seconds; and time 23.5 hours. After electrophoresis, gels were stained with ethidium bromide. Macrorestriction DNA banding patterns were digitized and analyzed using Molecular Analyst DNA Fingerprinting soft ware (BioRad). Patterns were compared and interpreted using the criteria of Tenover et al. [6].

Results

The results of PFGE identified five clonal types among our isolates. Twenty seven, 43% (27/62) showed a PFGE profile similar to the USA100 clone. Two isolates, 3% (2/62) showed a PFGE profile similar to the USA 200 clone. Twenty four isolates, 39% (24/62) showed a PFGE profile similar to the USA 300 clone. One isolate, 2% (1/62) showed a PFGE profile similar to USA 1100 clone. Eight isolates, 13% (8/62) could not be classified and were deemed unique.

The majority (24%,15/62) of isolates belonging to the USA 100 clonal type, were isolated from in-patients compared to 19% (12/62) from out-patients (24%). However, the majority of the USA 300 clonal type isolates, 29% (18/62), were isolated from out-patients compared to 9.6% (6/62) from in-patients (29%) (Table 1).

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Received July 22, 2015; **Accepted** October 09, 2015; **Published** October 12, 2015

Citation: Foggo-Osseyran A, Ross T, Rothwell L, DeSilva J, Tomkins K, et al. (2015) Molecular Typing of Methicillin-Resistant *Staphylococcus aureus* in Bermuda. J Medical Microbiol Diagnosis S5:006. doi:10.4172/2161-0703.S5-006

MRSA (PFGE) Type	USA 100	USA 200	USA 300	USA 1100	Unique
In-Patients	15 (24.1%)	2 (3.2%)	6 (9.6%)	1 (1.6%)	1 (1.6%)
Out-Patients	12 (19.3%)	0	18 (29%)	0	7 (11.2%)

Table 1: Breakdown of in-patients and out-patients MRSA clonal types by PFGE.

The percentages of USA 100 clonal types susceptible to trimethroprim-sulfamethoxazole, tetracycline, gentamicin, vancomycin and linezolid were 95%, 100%, 100%, 100% and 100% respectively. All of the USA 300 clonal types were susceptible to trimethroprim-sulfamethoxazole, tetracycline, gentamicin, vancomycin and linezolid. In addition, all of the unique clonal types were also susceptible to trimethroprim-sulfamethoxazole, tetracycline, gentamicin, vancomycin and linezolid.

Discussion

Molecular methods have been used to study the epidemiology of MRSA in hospitals and the community, with PFGE proving the most satisfactory method on the basis of its discriminatory ability and reproducibility [7]. In this study, we have confirmed the reports of other groups in the literature that USA 300 is a predominant clone in our community. It has been well established that USA 100 and 200 are the predominant clonal types in healthcare settings in the USA. However, in Asia and Oceania, USA 1100, a community strain, is the major clonal type [8]. There was no significant difference in the prevalence of the USA 100 clone in our hospital and the community. Also, both the USA 100 and USA 300 clones were susceptible to vancomycin, gentamicin and linezolid. In addition, the majority of both the USA 100 and 300 clones were susceptible to tetracycline and trimethroprim-sulfamethoxazole the preferred oral drugs for treating skin and soft tissue infections caused by MRSA. It is interesting to note that the USA 100 isolates in this study appear to be more susceptible than what is reported in the literature. Both HA-MRSA and CA-MRSA possess resistance to beta-lactam antimicrobial agents, conferred by the staphylococcal cassette chromosome (SCC) *mec* element [9]. However, CA-MRSA strains are typically less resistant to non-beta-lactam antimicrobial agents [10]. The Unique clonal types were found to be quite prevalent in the community, albeit their significance is yet to be determined. Both HA-MRSA and CA-MRSA have major consequences for infection control policies in hospitals and other healthcare facilities. In this study, CA-MRSA appears to cause healthcare-associated infections; an observation which has been mentioned by others in the literature [11]. Interestingly, a recent mathematical model developed by D'Agata et al. has postulated that USA 300 will become the predominant clone in US hospitals in the future and that it will probably manifest increased virulence relative to the USA 100 clone it will be displacing [12]. These findings highlight the need for infection control measures to prevent transmission within healthcare settings. Microbiological surveillance is an important tool to evaluate local infection control policy to assess which microorganisms are causative pathogens in healthcare settings. Furthermore, it has been suggested that surveillance per se probably results in a decrease of nosocomial infections as shown by the International Nosocomial Control Consortium (INICC) [13].

In conclusion, this study describes CA-MRSA USA 300 and HA-MRSA USA 100 clonal type emergence in Bermuda. In addition, it also demonstrates MRSA USA 300 clonal type transmission within healthcare settings. Empirical management with agents active against CA-MRSA should be considered for patients presenting with skin and soft tissue infections. Also, compliance to infection control policies and procedures when caring for patients with suspected CA-MRSA infections is critical for preventing transmission of these strains in healthcare settings.

Acknowledgments

The authors are most grateful to Lee Holder for excellent technical assistance.

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This article was originally published in a special issue, **Medical Genetics of Infections** handled by Editor(s). Dr. Joel Kenneth Weltman, University of Brown, USA