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Original article

Highly virulent M1 *Streptococcus pyogenes* isolates resistant to clindamycin

Résistance à la clindamycine chez des souches hypervirulentes de Streptococcus pyogenes *de type M1*

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Abstract

Context. – *Emm1*-type group A *Streptococcus* (GAS), or *Streptococcus pyogenes*, is mostly responsible for invasive infections such as necrotizing fasciitis (NF) and streptococcal toxic shock syndrome (STSS). The recommended treatment of severe invasive GAS infections is a combination of clindamycin and penicillin. Until 2012, almost all *emm1* isolates were susceptible to clindamycin.

Objectives. – We aimed to identify the phenotypic and genotypic characteristics of *emm*1 GAS clone resistant to clindamycin.

Methods. – GAS strains were characterized by *emm* sequence typing, detection of genes encoding pyrogenic exotoxins or superantigens. Cluster analysis was performed by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). Antibiotic susceptibility was assessed using disk diffusion and resistance genes were detected by PCR.

Results. – A total of 1321 GAS invasive isolates were analyzed between January 2011 and December 2012. The overall number of invasive isolates resistant to clindamycin was 52 (3.9%); seven of them were *emm1* isolates. All isolates had the same genomic markers: macrolide resistance due to the presence of the *erm*(B) gene, *emm* subtype 1.0, the same toxin or superantigen profile, PFGE pattern and sequence type.

Conclusion. – This is the first description of highly virulent GAS *emm1* isolates resistant to clindamycin in France. This article strengthens the need for monitoring the epidemiology of invasive GAS strains as they could lead to changes in treatment guidelines.

Keywords: Necrotizing fasciitis; Invasive infection; Clindamycin resistance; *Streptococcus pyogenes*; Virulence

Résumé

Contexte. – Les streptocoques du groupe A (SGA) ou *Streptococcus pyogenes* de génotype *emm1* (M1) sont le plus souvent responsables d'infections graves, en particulier de fasciites nécrosantes et de syndromes de choc toxique streptococcique. Le traitement recommandé des infections invasives graves à SGA associe la clindamycine à la pénicilline. Jusqu'en 2012, la quasi-totalité des isolats de SGA de type M1 était sensible à la clindamycine.

Objectifs. – Déterminer les caractères phénotypiques et génotypiques de souches de SGA de type M1 résistantes à la clindamycine.

Méthodes. – Les souches ont été caractérisées par séquençage du gène *emm*, détection de gènes codant pour des exotoxines ou superantigènes. L'analyse des clusters a été réalisée par électrophorèse en champ pulsé (ECP) et *multilocus sequence typing* (MLST). La sensibilité aux antibiotiques a été déterminée par diffusion en gélose et les gènes de résistance détectés par PCR.

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Résultats. – Mille trois cent vingt et un souches invasives de SGA ont été analysées de janvier 2011 à décembre 2012. Cinquante-deux (3,9 %) étaient résistantes à la clindamycine. Parmi ces souches, sept étaient de type M1. Tous les isolats possédaient les mêmes marqueurs génomiques : la présence du gène *erm* (B) responsable de la résistance aux macrolides, un génotype *emm* sous-type 1.0, les mêmes profils toxiniques, d'ECP et de MLST.

Conclusion. – Cette observation constitue la première description de SGA de type M1 responsables d'infections invasives résistants à la clindamycine en France. Ce travail souligne la nécessité de surveiller l'épidémiologie des isolats de SGA responsables d'infections invasives en raison des implications thérapeutiques potentielles qui pourraient en découler.

Mots clés : Fasciite nécrosante ; Infection invasive ; Résistance à la clindamycine ; *Streptococcus pyogenes* ; Virulence

1. Introduction

Streptococcus pyogenes (or group A *Streptococcus* [GAS]) is a human pathogen responsible for a wide variety of diseases ranging from pharyngitis to life-threatening infections such as necrotizing fasciitis (NF) or streptococcal toxic shock syndrome (STSS). In Europe, North America and Japan, *emm1*-type GAS remains one of the most frequent *emm* types. Compared with other *emm* types, it is responsible for many STSS and NF cases [1–6]. GAS isolates remain susceptible to betalactams, which is the standard treatment for GAS infections [7]. However, the use of clindamycin has been advocated in addition to penicillin to treat severe GAS infections, including NF or STSS. This may be explained by the agent's potent suppressor effect of virulence protein synthesis in bacteria and by its ability to inhibit GAS phagocytosis by neutrophils (regardless of the growth stage) [8,9]. Almost all *emm1* isolates are susceptible to clindamycin. We only identified one clindamycin-resistant *emm*1-type isolate due to the presence of the *erm*(B) gene in one of our former studies [3] of 1542 isolates responsible for invasive infections in adults between 2006 and 2010 in France.

It might, therefore, be difficult to treat severe invasive infections involving a clindamycin-resistant *emm*1-type isolate. Our aim was to study the occurrence of clindamycin-resistant isolates responsible for invasive infections between 2011 and 2012. We reported the clinical presentations associated with unusual *emm1*-type clindamycin-resistant GAS isolates.

2. Material and methods

2.1. Isolates and clinical data collection

The French national reference center for Streptococci (French acronym CNR-Strep) is responsible for the epidemiological surveillance of invasive GAS infections in France. The center prospectively collects GAS isolates from a network of 232 laboratories located in the 22 regions of France. Clinical data was obtained from questionnaires completed on a voluntary basis and sent back with isolates. Collected data included the patients' sex and date of birth, date and sample type, geographical area, and clinical presentations.

2.2. Case definition

GAS invasive infection was defined by the isolation of bacteria from a usually sterile site (e.g., blood, cerebrospinal fluid, bone, or joint fluid) or from samples obtained from a non-sterile site in combination with clinical signs of NF or STSS.

2.3. Microbiological methods

Isolates were confirmed to be *S. pyogenes* using morphological and growth characteristics including beta hemolysis on sheep blood agar, and grouping of carbohydrate with Lancefield group A specific antigen. All GAS isolates were *emm*-typed by sequencing the variable 5′-end of the *emm* gene and by comparing sequences with the database of the Center for disease control and prevention (CDC) [10] as described [11]. The detection of the genes encoding SpeA, SpeB, SpeC, Ssa, and SmeZ pyrogenic exotoxins or superantigens was performed by multiplex PCR and the *sic* gene was sequenced when necessary [3,12]. Cluster analysis was carried out by pulsed-field gel electrophoresis (PFGE) with *Sma*I restriction enzyme and interpreted according to Tenover criteria [13]. *sic* sequencing and multilocus sequence typing were performed as previously described [14,15]. The sequence type (ST) was assigned using the allelic profile and the MLST website [16]. Antibiotic susceptibility to penicillin G, amoxicillin, erythromycin, clindamycin, vancomycin, gentamicin, levofloxacin, moxifloxacin, and tetracycline was determined by the disk diffusion method according to the guidelines of the European committee on antimicrobial susceptibility testing (EUCAST). The double-disc (DD) diffusion test was used to determine macrolide resistance phenotype [17]. Erythromycin and clindamycin minimum inhibitory concentrations (MICs) were performed by Etest (bioMérieux). Macrolide resistance genes were detected by multiplex PCR according to a previously described procedure [18].

3. Results

A total of 1321 GAS invasive isolates were analyzed at the CNR-Strep between January 2011 and December 2012. During this period, the two *emm* types accounting for more than 40% of invasive isolates were *emm1* ($n = 359$, 27.2%) and