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PERFIL DE SUSCEPTIBILIDADE À CEFTAROLINA ENTRE ISOLADOS CLÍNICOS DE *Staphylococcus aureus*

Porto Alegre
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Trabalho de conclusão de curso de especialização apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Especialista em Microbiologia Clínica.

Orientador: Prof. Dr. Leandro Reus Rodrigues Perez.

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RESUMO

Staphylococcus aureus é um agente comumente encontrado em pneumonias. A prevalência de isolados de *S. aureus* resistente à meticilina (MRSA) vem aumentando, o que torna o tratamento cada vez mais desafiador. Infecções por MRSA estão associadas com maior índice de mortalidade quando comparadas a isolados de *S. aureus* sensíveis à meticilina (MSSA). O antimicrobiano de escolha para tratamento de infecções por MRSA é a vancomicina, porém já existem relatos de isolados com susceptibilidade reduzida, e, raros casos de resistência à vancomicina. A ceftarolina é uma cefalosporina de quinta geração recentemente introduzida na terapêutica e que possui ação anti-estafilocócica, inclusive contra isolados de MRSA. Apesar da eficácia da ceftarolina ser comprovada para grande parte dos isolados de MRSA, já existem relatos de resistência. Com isso, este estudo teve o objetivo de avaliar o perfil de suscetibilidade à ceftarolina de isolados de *S. aureus* através da concentração inibitória mínima (CIM). O trabalho foi desenvolvido utilizando 248 isolados de *S. aureus* provenientes de amostras de trato respiratório, sendo 124 MRSA e 124 MSSA selecionadas aleatoriamente entre agosto e dezembro de 2019 em três hospitais de Porto Alegre. Do total de isolados, apenas 2 MRSA apresentaram resistência a ceftarolina (1,6%), enquanto todos isolados de MSSA apresentaram susceptibilidade à ceftarolina. Pode-se observar que as CIM diferem entre isolados de MRSA (0,5/0,75 mg/dL) e MSSA (0,38/0,5 mg/dL). Em relação ao perfil de susceptibilidade dos outros antimicrobianos avaliados, foi possível observar que isolados de MRSA apresentam maior resistência a ciprofloxacina e eritromicina, enquanto isolados de MSSA apresentam maior resistência a clindamicina e gentamicina. Não foi observada diferença sulfametoxazol/trimetoprima entre isolados de MSSA e MRSA. Desta forma, a ceftarolina apresentou potente atividade *in vitro* contra isolados S. aureus obtidos de amostras de trato respiratório, incluindo MRSA. No entanto as CIM superiores encontradas em isolados de MRSA quando comparados aos de MSSA nos mostram a importância do constante monitoramento do perfil de susceptibilidade da ceftarolina.

Palavras-chave: Ceftarolina. *Staphylococcus aureus*. MRSA. Resistência antimicrobiana. Epidemiologia.

ABSTRACT

Staphylococcus aureus is a common cause of pneumonia. Infections with meticillin-resistant S. aureus (MRSA) seriously impact treatment outcomes and increase mortality rates when compared infections caused by meticillin-susceptible *S. aureus* (MSSA). The antimicrobial of choice for treating MRSA infections is vancomycin. Ceftaroline is a fifth-generation cephalosporin with antimicrobial activity against multidrug-resistent gram-positive pathogens, including MRSA isolates. Although the effectiveness of ceftaroline has been proven for most MRSA isolates, there are already reports of resistance. The aim of this study was to evaluate the ceftaroline minimum inibitory concentration (MIC) distribution and to determine the sensibility patern of other agentes against S. *aureus* isolates recovered from clinical respiratory specimes. A set of 248 S. aureus isolates, including 124 MRSA and 124 MSSA, were randomly selected for this study. Of the isolates, only 2 (1,6%) MRSA isolates would be resistant to ceftaroline, while all MSSA isolates would be characterized as susceptible. Moreover, among our selected isolates the ceftaroline MIC value differs between MRSA (0.5 / 0.75 mg / dL) and MSSA (0.38 / 0.5 mg / dL). Regarding the susceptibility profile to other agents, it was possible to observe that MRSA isolates were more resistant to ciprofloxacin and erythromycin, while MSSA isolates presented to be more resistant to clindamycin and gentamycin. No difference was observed for trimethoprim/sulfamethoxazole among MSSA and MRSA isolates. Ceftaroline presented potent *in vitro* activity against respiratory *S. aureus* isolates, including MRSA. However, the higher MICs among MRSA isolates in comparison with MSSA isolates show us the importance of constantly monitoring the susceptibility profile of ceftaroline.

Keywords: Ceftaroline. *Staphylococcus aureus*. MRSA. Antimicrobial resistance. Surveillance.

SUMÁRIO

1 INTRODUÇÃO

Staphylococcus aureus é um patógeno oportunista que constitui a microbiota normal do ser humano, habitando pele e mucosas nasais de indivíduos saudáveis. Apesar de fazer parte da microbiota normal, pode causar infecções em diversos tecidos, que variam desde infecções leves como foliculites e abcessos cutâneos, a infecções graves como endocardites, pneumonias e bacteremias (Balasubramanian et al. 2017, Lakhundi & Zhang 2018, Sakr et al. 2018). *S. aureus* é um patógeno altamente adaptável, ou seja, pode ser colonizador de vários sítios, incluindo os abióticos como próteses cirúrgicas, cateteres e demais superfícies (Balasubramanian et al. 2017).

O tratamento de infecções causadas por *S. aureus* torna-se cada vez mais desafiador devido à alta prevalência de isolados resistentes a meticilina. As infecções por cepas de *S. aureus* resistentes a meticilina (MRSA) estão associadas a um maior índice de mortalidade quando comparadas a cepas sensíveis à meticilina (MSSA) (Balasubramanian et al. 2017; Sakr et al. 2018). Infecções por MRSA impactam diretamente no aumento do tempo de permanência hospitalar, bem como no aumento dos custos de internação (Lakhundi and Zhang 2018). No ano de 2017, a Organização Mundial de Saúde (OMS) publicou um documento citando uma lista de microrganismos que necessitam de atenção prioritária no contexto da resistência aos antimicrobianos, e isolados de MRSA estavam entre os microrganismos citados como alta prioridade (WHO, 2017). Em 2016, segundo dados da Agência Nacional de Vigilância Sanitária (ANVISA), o *S. aureus* foi o terceiro agente etiológico (14,1%) de infecções de corrente sanguínea associadas ao cateter venoso central nas unidades de terapia intensivas (UTI) brasileiras, e na região sul do Brasil, ele é o segundo patógeno mais prevalente. Destes dados analisados, cerca de 63% eram MRSA, representando um aumento de 10% quando comparado aos mesmos dados do ano de 2012 (ANVISA, 2016).

A meticilina é uma penicilina semissintética desenvolvida na década de 60 como opção terapêutica para isolados produtores de β-lactamases. Entretanto, cerca de um ano após o início do seu uso já apareciam os primeiros isolados resistentes (Lakhundi & Zhang 2018). O mecanismo de resistência à meticilina se dá pela aquisição do gene mecA, que está localizado no cassete cromossômico estafilocócico SCCmec. O gene mecA é responsável pela codificação de proteínas ligadoras de penicilina anômalas (PBP2a) que possuem baixa

afinidade aos β-lactâmicos, impedindo que estes se liguem a parede celular bacteriana (Munita et al. 2015).

As cepas de MRSA eram vinculadas aos hospitais (HA-MRSA), entretanto, na década de 1980 começaram a surgir cepas de MRSA associadas à comunidade (CA-MRSA), criando reservatórios em ambos tipos de ambientes. Inicialmente as cepas de CA-MRSA apresentavam um perfil com menor resistência, o que permitia uma fácil diferenciação entre as cepas de HA-MRSA e CA-MRSA. Atualmente, com o aumento da prevalência de CA-MRSA, este perfil vem se modificando e sendo responsável por surtos nosocomiais em estabelecimentos de saúde (Lakhundi and Zhang 2018).

O tratamento de primeira escolha para infecções causadas por MRSA é a vancomicina, antimicrobiano desenvolvido há mais de 50 anos. Existem relatos de isolados de *S. aureus* com susceptibilidade reduzida à vancomicina (VISA) bem como relatos, em pequenos números, de isolados com resistência à vancomicina (VRSA), particularmente associadas à aquisição do gene *van*A, mecanismo bastante associado aos isolados de *Enterococcus* spp. (Arias et al. 2017; Munita et al. 2015).

Em 2014 foi aprovada pela ANVISA a ceftarolina, uma cefalosporina de quinta geração que têm sua ação semelhante aos demais β-lactâmicos, com afinidade de ligação as PBP's, inibindo a síntese de parede celular. O que diferencia a ceftarolina dos demais β-lactâmicos é sua afinidade também pela PBP2a, fazendo com que esta apresente atividade anti-MRSA (Batista 2015, Gil Romero & Gómez-Garcés 2019, Tenorio-Abreu et al. 2015).

Considerando as informações apresentadas e o fato de a ceftarolina ter apenas seis anos de uso, sendo considerada uma opção terapêutica relativamente nova, este estudo se propõe a avaliar o perfil de sensibilidade desta droga em isolados clínicos de *S. aureus* por meio da determinação das concentrações inibitórias mínimas.

1.1 OBJETIVOS

1.1.1 Objetivo geral

Estabelecer o perfil de susceptibilidade do *Staphylococcus aureus* frente à ceftarolina por meio da determinação da concentração inibitória mínima.

1.1.2 Objetivos específicos

a) Comparar o nível de susceptibilidade das cepas de MRSA e MSSA frente à ceftarolina;
b) Estabelecer o perfil de susceptibilidade à outras drogas anti-estafilocócicas, tais como clindamicina, eritromicina, gentamicina e sulfametoxazol/trimetoprima.

2 ARTIGO CIENTÍFICO

Letter to the Editor

A snapshot survey of antimicrobial susceptibility among respiratory *Staphylococcus aureus* isolates: focus on ceftaroline

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KEYWORDS: Ceftaroline; *Staphylococcus aureus*; MRSA; antimicrobial resistance; surveillance

Staphylococcus aureus is a common cause of nosocomial and community-acquired pneumonia. Infections with methicillin-resistant *S. aureus* (MRSA) seriously impact treatment outcomes and increase mortality rates when compared to respiratory infections caused by methicillin-susceptible *S. aureus* (MSSA).[1]

Methicillin-resistance is due to the production of an additional penicillin-binding protein (PBP), called PBP 2′ or PBP 2a. This PBP2a, encoded by the *mec*A gene, confers resistance to virtually all β-lactam agents and their derivatives because of its low binding affinity.[2]

Ceftaroline, the active compound of the pro-drug ceftaroline fosamil, is a broad-spectrum cephalosporin with bactericidal activity against multidrug-resistant gram-positive organisms. Ceftaroline has been approved by the Food and Drug Administration (FDA) for treating acute bacterial skin infection, including those MRSA-associated infections, and community-acquired bacterial pneumonia.[3]

Although ceftaroline is effective against most MRSA isolates, resistance has already been documented.[4] Despite the attributed mechanism for this is controversial and poorly understood so far, it is crucially important its early detection and monitoring.

The aim of this study was to evaluate the ceftaroline minimum inhibitory concentration (MIC) distribution and to determine the susceptibility pattern of other agents against *S. aureus* isolates recovered from clinical respiratory specimens, for which ceftaroline would be a therapeutic indication.

A set of 248 *S. aureus* isolates, including 124 MRSA and 124 MSSA, were randomly selected for this study. They were collected between August 1st and December 2019 from clinical respiratory specimens (sputum, endotracheal aspirate and bronchoalveolar lavage), of inpatients or those seeking emergency service of three different hospitals in Porto Alegre, Southern Brazil. We do not include colonized patients.

Bacterial identification was made using phenotypic tests such as catalase and coagulase tests and MALDI-TOF (bioMérieux, Marcy I'Etoile, France) when necessary. Ceftaroline MICs were determined by Etest strips (bioMérieux, Marcy I'Etoile, France).

Cefoxitin susceptibility, used as surrogate marker for methicillin resistance mediated by *mec*A gene, as well as susceptibility to clindamycin, ciprofloxacin, erythromycin, gentamycin and trimethoprim/sulfamethoxazole (TMP/SMX) were determined by disc-diffusion test and interpreted according to EUCAST breakpoints.[5] *Staphylococcus aureus* ATCC 29213 was used as the quality control isolate.

According to EUCAST breakpoints for *S. aureus* pneumonia ($\leq$1.0 and >1.0 mg/L for susceptible and resistant, respectively), only 2 (1.6%; 2/124) MRSA isolates would be resistant to ceftaroline while all MSSA isolates would be characterized as

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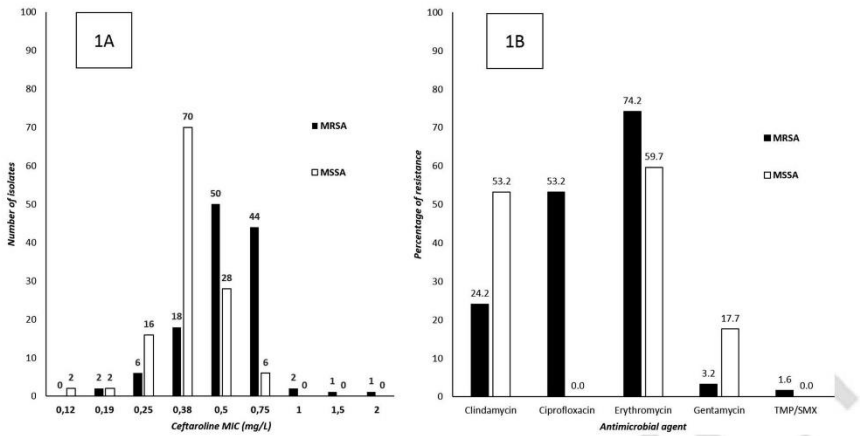

Figure 1 Ceftaroline MICs distribution (1 A) and antimicrobial susceptibility profile among 124 MRSA and 124 MSSA isolates (1B). TMP/SMX is trimethoprim/sulfamethoxazole.

susceptible. Moreover, among our selected isolates, the ceftaroline $MIC_{50/90}$ value differs between MRSA and MSSA (0.5/0.75 mg/L and 0.38/0.5 mg/L, in that order). Ceftaroline MICs distribution for all isolates included in this study is shown in Figure 1A.

Regarding the susceptibility profile to other agents, MRSA isolates were more resistant to ciprofloxacin and erythromycin while MSSA isolates presented to be more resistance to clindamycin (including those induced by *erm* gene) and gentamycin. No difference was virtually observed for TMP/SMX among MRSA and MSSA isolates (Figure 1B).

Ceftaroline is a new parenteral cephalosporin with antimicrobial activity against multidrug-resistant gram-positive bacteria, including MRSA, those with reduced susceptibility to vancomycin and *Streptococcus pneumoniae* with reduced susceptibility to penicillins, erythromycin, and fluoroquinolones.[6]

Ceftaroline can overcome inactivation due to PBP2a production since it retains high affinity, making it activity usually maintained against MSSA or MRSA isolates. On the other hand, ceftaroline may be impacted by a modified PBP2a as result of a genetic mutation in allosteric site, essential for binding the drug.[4]

High-level of PBP expression, others than PBP2a such as PBP4,[7] appears to confer on the face of a persistent stimulus (β-lactam exposure, for example) a major ability to reduce the susceptibility to this drug (Figure 1A). Thus, more attention for the use of it in infections caused by MRSA can be required.

It is important to note some other points: MSSA isolates may also present any level of resistance, involving overexpression of other PBPs than PBP2a, similarly to MRSA; second, a higher $MIC_{50/90}$ (although into the susceptibility range) among MRSA when compared to MSSA isolates may represent a mechanism of adaptation or tolerance.

Surveillance data in some regions of Latin America and the Asia Pacific have reported ceftaroline MIC_{90} values of 2.0 mg/L for *S. aureus* and rare isolates with ceftaroline MICs of 4.0 mg/L.[8,9]

Ceftaroline have been supported as a valuable option to treat patients with pneumonia. In a systematic review and meta-analysis to retrieve both experimental and observational studies, a substantial and critical analysis provided a high efficacy and effectiveness of this antimicrobial agent against *S. pneumoniae* and *S. aureus*, as well as its safety and tolerability.[10]

Results from the AWARE (Assessing Worldwide Antimicrobial Resistance Evaluation) program, in the USA, showed that ceftaroline potentially offers the inherent benefits of β-lactam therapy, even in monotherapy, in the treatment of community-acquired bacterial pneumonia, including those caused by MRSA.[11]

Although no molecular characterization was performed, the antimicrobial susceptibility profile from MRSA isolates suggests the presence of SCC*mec* type I and IV, characterized by TMP/SMX susceptibility, which possibly could displace the SCC*mec* dominant in Southern Brazil past years.[12] Curiously, MSSA isolates shown be more resistant for some antibiotics (clindamycin and gentamycin, for example) than MRSA isolates.

In conclusion, ceftaroline presented a potent *in vitro* activity against respiratory *S. aureus*

isolates, including MRSA. However, a higher ceftaroline MICs among MRSA in comparison with MSSA isolates was observed. Ceftaroline may represent a valuable option for treatment of staphylococcal pneumonia, as seen here and in other studies, but susceptibility levels should be strictly monitored to avoid resistance development to this drug.

Disclosure statement

No potential conflict of interest was reported by the authors.

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3.CONCLUSÃO E PERSPECTIVAS

Os resultados demonstraram que a suscetibilidade à ceftaroline é ainda favorável, conforme distribuição da CIM demonstrada entre os isolados de MRSA e MSSA. Entretanto, o perfil de susceptibilidade às demais drogas anti-estafilocócicas foram diferentes entre os isolados de MRSA e MSSA e, desta forma, o conhecimento sobre a distribuição da susceptibilidade entre isolados de *S. aureus* é necessário para a seleção da melhor estratégia terapêutica. Constante monitoramento dos perfis de susceptibilidade compõem as boas práticas de uso de antimicrobianos (antimicrobial stewardship), evitam a disseminação da resistência bacteriana e promovem melhores desfechos clínicos aos pacientes.

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ANEXO A – NORMAS DE SUBMISSÃO DO JOURNAL OF CHEMOTHERAPY

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- Should be no more than 9000 words, inclusive of the abstract, tables, references, figure captions.
- Should contain an unstructured abstract of 150 words.
- Should contain between 6 and 8 **keywords**. Read making your article more discoverable, including information on choosing a title and search engine optimization.
- Papers on either Antimicrobial or Anticancer topics are accepted.

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- Should be written with the following elements in the following order: title page; abstract; keywords; main text introduction, materials and methods, results, discussion; acknowledgments; declaration of interest statement; references; appendices (as appropriate); table(s) with caption(s) (on individual pages); figures; figure captions (as a list)
- Should be between 1200 and 1500 words , inclusive of the abstract.
- Should contain an unstructured abstract of 150 words.
- Should contain between 6 and 8 **keywords**. Read making your article more discoverable, including information on choosing a title and search engine optimization.
- Authors may add either 2 tables or figures and no more than 20 references.

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- Should be written with the following elements in the following order: title page; figures; figure captions (as a list)
- Should be between 1 and 1.5 pages .
- Letters to the editor should contain only either one figure or table, no sections (e.g. introduction, material, methods) and no more than 10 references.

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