**Control/Tracking Number:**2022-A-3578-MICROBE  
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**Frequency Of Cefazolin Inoculum Effect Among Bacteremic Methicillin-susceptible *Staphylococcus Aureus*, Is It A Cause For Concern?**  
  
  
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**Abstract:**  
Background: Recent studies suggest that cefazolin (Cz) has clinical efficacy similar to isoxazolyl penicillins for the treatment of methicillin-susceptible *Staphylococcus aureus* (MSSA) bacteremia with a lower rate of adverse events and greater ease of administration. However, the Cz inoculum effect (CzIE), mediated by staphylococcal beta-lactamases, could limit the therapeutic efficacy of Cz. In this study the presence of CzIE was analyzed in 55 bloodstream MSSA isolates recovered consecutively from 55 patients in a Spanish hospital during 2020-2021. Methods: The identification of the isolates was performed by MALDI-TOF. Cz MICs were determined at standard (105 CFU/mL) and high (107 CFU/mL) inoculum by broth microdilution. The CzIE was defined as an increase of MIC to ≥16 mg/L when tested at high inoculum. *S. aureus* ATCC 29213, a producer of BlaZ type A beta-lactamase lacking the CzIE, was used as control strain.The characterization of the *blaZ*gene was performed in all isolates by PCR (using new designed primers to amplify the complete gene) and sequencing; BlaZvariants and allotypes were stablished according to Carvajal et al (AAC 2020; 64:e02511-19). Results: The Cz MIC90 for all isolates when tested at standard inoculum was 1 mg/L. The overall prevalence of the CzIE was 20% (11/55). Among the 55 BlaZsequences, type B was the most predominant beta-lactamase (n=32; 58%), followed by type A (n=11; 25%), and type C (n=9; 16%). Most isolates with type A (10/14; 71.4%) showed CzIE; however, none of the type B isolates, and only one isolate with type C beta-lactamase showed CzIE. We found 10 allotypes in BlaZ, being BlaZ-1, -2, -3 and -7 predominants (83%). A single allotype, designated BlaZ-2, was present in 72.7% (8/11) of isolates showing CzIE. All BlaZ-2 isolates presented three critical amino acid substitutions (A9V, E112A, and G145E). Two other allotypes (BlaZ-3 and BlaZ-7) were associated with a lack of the CzIE. Conclusion: Among recent bloodstream MSSA isolates, the prevalence of the CzIE was 20% and was mostly associated with type A beta-lactamase. The presence of type A beta-lactamase could predict the CzIE among MSSA clinical isolates.  
**Acknowledgments/ References:**  
Carvajal LP, et al. Novel Insights into the Classification of Staphylococcal β-Lactamases in Relation to the Cefazolin Inoculum Effect. Antimicrob Agents Chemother. 2020;64(5):e02511-19.  
  
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