J Antimicrob Chemother 2013; **68**: 2773–2778
doi:10.1093/jac/dkt254 Advance Access publication 21 June 2013

Journal of Antimicrobial Chemotherapy

Cefazolin high-inoculum effect in methicillin-susceptible *Staphylococcus aureus* from South American hospitals

Sandra Rincón[1], Jinnethe Reyes[1,2], Lina Paola Carvajal[1], Natalia Rojas[1], Fabián Cortés[3], Diana Panesso[1,2], Manuel Guzmán[4], Jeannete Zurita[5,6], Javier A. Adachi[7], Barbara E. Murray[2,8], Esteban C. Nannini[9] and Cesar A. Arias[1,2]*

[1]Molecular Genetics and Antimicrobial Resistance Unit, Universidad El Bosque, Carrera 7B Bis No. 132-11, Bogotá, Colombia; [2]Department of Internal Medicine, Division of Infectious Diseases, University of Texas Medical School at Houston, 6431 Fannin Street, MSB 2.112, Houston, TX 77030, USA; [3]Division de Investigaciones, Universidad El Bosque, Carrera 7B Bis No. 132-11, Bogotá, Colombia; [4]Centro Médico Caracas, Av. Eraso, Plaza El Estanque, Urb. San Bernardino, Caracas, Venezuela; [5]Facultad de Medicina, Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre 1076 y Roca, Quito, Ecuador; [6]Hospital Vozandes, Q Villalengua Oe2-37 y Av. 10 de Agosto, Quito, Ecuador; [7]Department of Infectious Diseases, Infection Control & Employee Health University of Texas, MD Anderson Cancer Center, 1515 Holcombe Blvd, Unit 1460, Houston, TX 77030, USA; [8]Department of Microbiology and Molecular Genetics, University of Texas Medical School at Houston, 6431 Fannin Street, MSB 2.112, Houston, TX 77030, USA; [9]Universidad Nacional de Rosario and Sanatorio Británico, Paraguay 40, Rosario 2000, Santa Fe, Argentina

*Corresponding author. Division of Infectious Diseases, University of Texas Medical School at Houston, 6431 Fannin St, MSB 2.112, Houston, TX 77030, USA. Tel: +1-713-500-6738; Fax: +1-713-500-5495; E-mail: cesar.arias@uth.tmc.edu

Received 26 March 2013; returned 17 April 2013; revised 23 May 2013; accepted 24 May 2013

Objectives: Clinical failures with cefazolin have been described in high-inoculum infections caused by methicillin-susceptible *Staphylococcus aureus* (MSSA) producing type A β-lactamase. We investigated the prevalence of the cefazolin inoculum effect (InE) in MSSA from South American hospitals, since cefazolin is used routinely against MSSA due to concerns about the *in vivo* efficacy of isoxazolyl penicillins.

Methods: MSSA isolates were recovered from bloodstream ($n=296$) and osteomyelitis ($n=68$) infections in two different multicentre surveillance studies performed in 2001–02 and 2006–08 in South American hospitals. We determined standard-inoculum (10^5cfu/mL) and high-inoculum (10^7 cfu/mL) cefazolin MICs. PFGE was performed on all isolates that exhibited a cefazolin InE. Multilocus sequence typing (MLST) and sequencing of part of *blaZ* were performed on representative isolates.

Results: The overall prevalence of the cefazolin InE was 36% (131 isolates). A high proportion (50%) of MSSA isolates recovered from osteomyelitis infections exhibited the InE, whereas it was observed in 33% of MSSA recovered from bloodstream infections. Interestingly, Ecuador had the highest prevalence of the InE (45%). Strikingly, 63% of MSSA isolates recovered from osteomyelitis infections in Colombia exhibited the InE. MLST revealed that MSSA isolates exhibiting the InE belonged to diverse genetic backgrounds, including ST5, ST8, ST30 and ST45, which correlated with the prevalent methicillin-resistant *S. aureus* clones circulating in South America. Types A (66%) and C (31%) were the most prevalent β-lactamases.

Conclusions: Our results show a high prevalence of the cefazolin InE associated with type A β-lactamase in MSSA isolates from Colombia and Ecuador, suggesting that treatment of deep-seated infections with cefazolin in those countries may be compromised.

Keywords: inoculum effect, bloodstream infections, osteomyelitis

Introduction

Staphylococcus aureus is a leading cause of hospital- and community-acquired infections.[1] The agents of choice for severe infections caused by methicillin-susceptible *S. aureus* (MSSA) are the isoxazolyl penicillins (such as oxacillin, nafcillin and flucloxacillin). These compounds are widely used in the hospital setting for the treatment of severe MSSA infections. However, due to the need for frequent administration, clinicians tend to switch to cephalosporins such as cefazolin in order to continue therapy in

an outpatient setting.[2,3] However, the use of cefazolin in the treatment of deep-seated MSSA infections in which high bacterial inocula are present may be compromised[4] by the production of certain types of staphylococcal β-lactamases that are capable of degrading cefazolin. Four different types (A–D) of staphylococcal β-lactamase enzymes have been characterized based on their substrate specificity and amino acid sequence,[5,6] some of which are able to hydrolyse cefazolin at significant rates.[5] Under inducing conditions (e.g. the presence of a β-lactam antibiotic), *S. aureus* isolates can produce large quantities of β-lactamase, a phenomenon that may compromise the effectiveness of cefazolin[7] in infections where a high inoculum is present (e.g. endocarditis, osteomyelitis and undrained abscesses).[8,9] Under these circumstances, cefazolin degradation may occur (depending on the substrate specificity of the enzyme), leading to decreased concentrations of the antibiotic at the site of infection, with the potential to produce therapeutic failures.[8,10,11] Indeed, cefazolin degradation and inactivation has been associated with clinical failures in high-inoculum infections with *S. aureus* strains producing type A β-lactamase.[4,11,12]

The inoculum effect (InE) has been defined as a significant rise in the cefazolin MIC when the bacterial inoculum size is increased to 10^7cfu/mL (instead of the standard 10^5cfu/mL).[8,9,13] Previous studies in the USA have reported that the prevalence of MSSA isolates exhibiting the cefazolin InE ranged from 19% to 27%.[11,14] Cefazolin (instead of oxacillin or its derivatives) is frequently used in South America as the 'therapy of choice' for the treatment of severe MSSA infections, since the isoxazolyl penicillins are not commercially available in certain countries, generic derivatives are thought to be clinically inferior to the innovator compound[15] or because of a more favourable dosing schedule as the availability of efficient outpatient systems for intravenous administration is limited in some Latin American countries. However, the prevalence of the cefazolin InE among MSSA isolates recovered from invasive infections in South America is unknown. In this study, we sought to characterize the frequency of the cefazolin InE among MSSA isolates recovered from invasive infections (bacteraemia and osteomyelitis) in two previous prospective multicentre surveillance studies performed in South American hospitals. Additionally, we performed molecular characterization of representative MSSA isolates and typing of β-lactamase enzymes of MSSA isolates exhibiting such a phenotype. A high prevalence of InE was found in MSSA recovered from Ecuadorian and Colombian hospitals.

Materials and methods

Bacterial isolates and species identification

A total of 364 MSSA clinical isolates were included in this study. The organisms were recovered from two previous prospective multicentre clinical studies.[16,17] The first study was performed in 2001–02 and encompassed 15 tertiary care centres in five Colombian cities.[16] The second study was carried out in 2006–08 and included 32 tertiary hospitals from four South American countries, including Colombia (22 hospitals), Ecuador (5 hospitals), Peru (3 hospitals) and Venezuela (2 hospitals).[17] The isolates were collected from individual patients presenting with bloodstream infections ($n=296$) or osteomyelitis ($n=68$) (cultures directly from bone tissue after biopsy). Confirmation of species and presence of the *mecA* gene in all isolates was performed by a multiplex PCR assay.[18]

Susceptibility testing

Cefazolin (Sigma Chemicals, St Louis, MO, USA) MICs were determined using a broth microdilution method with cation-adjusted Mueller–Hinton broth II (Becton Dickinson) at standard inoculum (10^5cfu/mL) following the CLSI recommendations.[13] High-inoculum MICs using 10^7cfu/mL were evaluated simultaneously in order to determine whether there was a cefazolin InE, as previously described.[11] MICs were determined at 24 h by two different researchers. The inocula were confirmed by performing colony counts. The high-inoculum effect was defined as a cefazolin MIC ≥16 mg/L at high inoculum and ≤8 mg/L (susceptible) at standard inoculum.[11] The *S. aureus* strains included as controls were as follows: TX0117, a high-level producer of type A β-lactamase,[4] which exhibits the high-inoculum effect; *S. aureus* ATCC 29213, a producer of small amounts of type A β-lactamase; and *S. aureus* ATCC 25923, a β-lactamase-negative strain.[4,11]

Molecular typing

Pulsed-field gel electrophoresis (PFGE) was performed on all isolates that exhibited a cefazolin InE, as previously described.[17,19] *S. aureus* NCTC 8325 was used as the control for molecular size fragments. Methicillin-resistant *S. aureus* (MRSA) circulating in South American hospitals and strains from pandemic clones were included as comparators for PFGE banding analysis and involved representatives of the community-associated MRSA USA300 Latin American variant clone (ComA), USA300, Chilean, Brazilian, Iberian, Pediatric (NRSA 387) and New York/Japan (NRSA 382) clones. Banding patterns were analysed using Gel ComparII software version 4.01 (Applied Maths, Belgium). The Dice coefficient was calculated using a tolerance of 0.5 and dendrograms were constructed by the unweighted pair group method. The percentage of similarity to define a pulsotype was ≥75% and letters were used to designate them. Multilocus sequence typing (MLST) was performed for selected isolates from the most prevalent pulsotypes and highest MICs, as previously described.[20]

Detection of β-lactamase gene (*blaZ*) and sequencing

The β-lactamase gene *blaZ* was detected by PCR in all isolates exhibiting the InE, following the methodology previously described by Martineau *et al*.[18] Sequencing of part of *blaZ* (a 355 bp fragment inside of the *blaZ* gene in order to identify putative amino acid differences at residues 128–216)[21] was performed using representative isolates of the most prevalent PFGE types from blood and osteomyelitis, according to a methodology described previously.[11] Analysis and sequence alignments of *blaZ* sequences were performed using the BLAST tool from NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis

The MIC means, ranges, MIC_{50} and MIC_{90} at standard and high inocula were calculated. Comparison between InEs among countries was performed using the *Z*-test for difference between proportions. ANOVA and non-parametric statistics (Wilcoxon rank-sum test and Mann–Whitney *U*-test) were used to compare the cefazolin standard-inoculum versus high-inoculum MIC by clinical source and country. The *Z*-test for differences between proportions was used to compare frequencies of the cefazolin high-inoculum effect in Colombia from the two multicentre studies. The χ^2 test of independence or homogeneity or Fisher exact tests were used to compare the frequency of the InE between countries. The tests were considered statistically significant if the *P* value was <0.05. Statistical analysis was performed using Stata software (version 10, Stata Corporation, College Station, TX, USA).

Results

Prevalence of cefazolin InE in MSSA clinical isolates from South America

A total of 364 MSSA recovered from bloodstream infections and osteomyelitis in two multicentre surveillance studies in South American hospitals were evaluated for the cefazolin InE. All isolates were susceptible to cefazolin at standard inoculum (MICs < 8 mg/L, with the highest MIC value of 2 mg/L). A marked and statistically significant increase in the MICs was observed in 131 (36%) isolates when a high inoculum was used (97 and 34 recovered from blood and bone, respectively) (Table 1). Of note, 50% (34 out of 68) of MSSA isolates recovered from osteomyelitis exhibited the InE, whereas it was documented in 33% of bloodstream isolates (97 out of 296). Among these 131 isolates, the MIC range at the standard inoculum was 0.25 to ≤2 mg/L and increased to 16 to ≤512 mg/L at the high inoculum (Table 1). Of note, 28 MSSA isolates showed very high cefazolin MICs at the high inoculum (≥128 mg/L) (18 from blood and 10 from osteomyelitis).

Table 1. Cefazolin MIC_{50}, MIC_{90}, range and IQR (mg/L) at standard inoculum and high inoculum among 131 MSSA isolates demonstrating the InE by clinical source

Clinical source	Parameter	Cefazolin MICs (mg/L)		*P* value[a]
		SI	HI	
Bloodstream (*n* = 97)	mean	0.65	87.75	<0.001
	95% CI	0.59–0.70	64.1–111.3	
	range	0.25–2	16–512	
	IQR	0.5–1.0	16–64	
	MIC_{50}	0.5	64	
	MIC_{90}	1.0	64	
Osteomyelitis (*n* = 34)	mean	0.80	120.94	<0.001
	95% CI	0.65–0.95	70.0–121.8	
	range	0.5–2	16–512	
	IQR	0.5–1	32–256	
	MIC_{50}	0.5	64	
	MIC_{90}	1.0	256	
Total of isolates (*n* = 131)	mean	0.69	96.36	<0.001
	95% CI	0.63–0.74	74.4–118.29	
	range	0.25–2	16–512	
	IQR	0.5–1.0	16–64	
	MIC_{50}	0.5	64	
	MIC_{90}	1.0	256	

SI, standard inoculum (~10^5 cfu/mL); HI, high inoculum (~10^7 cfu/mL); MIC_{50}, cefazolin concentration at which 50% of *S. aureus* isolates were inhibited; MIC_{90}, cefazolin concentration at which 90% of *S. aureus* isolates were inhibited.
[a]Statistically significant differences were calculated by Mann–Whitney *U*-test comparing mean MICs at HI and SI.

Table 2 shows the distribution of the cefazolin InE by country and surveillance time. In the first surveillance study, performed in 2001–02, which included 15 Colombian hospitals, a high proportion of isolates exhibited the cefazolin InE (46%) (45% of bloodstream MSSA isolates and 57% of osteomyelitis isolates). In the second and more comprehensive surveillance study, performed between 2006–08, which included hospitals in Colombia, Venezuela, Peru and Ecuador, the overall prevalence of the cefazolin InE in MSSA recovered from deep-seated infections was 34%. When the data were analysed by country of origin, Ecuador had the highest prevalence of MSSA isolates exhibiting the cefazolin InE, with almost half (45%) of the MSSA isolates showing this phenotype (40% and 56% of bloodstream and osteomyelitis isolates, respectively). The most striking finding was that >60% of MSSA isolates recovered from osteomyelitis in Colombia during this surveillance study exhibited the cefazolin InE. The lowest prevalence of the cefazolin InE was observed in Peru and Venezuela (26% and 29%, respectively).

Molecular characterization of MSSA isolates exhibiting the cefazolin InE

PFGE was performed in all 131 MSSA isolates exhibiting the InE. We were able to differentiate 39 different pulsotypes among bloodstream MSSA, whereas 16 pulsotypes were identified in isolates recovered from osteomyelitis. In MSSA isolates from blood, two pulsotypes were the most frequent (designated Q and O), encompassing 20% and 10% of isolates, respectively. On the other hand, 33% of MSSA isolates recovered from osteomyelitis belonged to

Table 2. Frequency of cefazolin InE in MSSA from South American hospitals

Multicentre study	Country	Frequency of InE				
		blood		osteomyelitis		
		no. isolates evaluated	no. (%) isolates with InE	no. isolates evaluated	no. (%) isolates with InE	total isolates evaluated/MSSA with InE (%)
2001–02	Colombia	49	22 (45)	7	4 (57)	56/26 (46)
2006–08	Colombia	150	46 (31)	16	10 (63)	166/56 (34)
	Ecuador	40	16 (40)	18	10 (56)	58/26 (45)
	Peru	39	10 (26)	0	0	39/10 (26)
	Venezuela	18	3 (17)	27	10 (37)	45/13 (29)
	total	247	75 (30)	61	30 (49)	308/105 (34)

InE, inoculum effect.

two major pulsotypes (designated OB and OF, with 18% and 15% of isolates, respectively). Of note, no genetic relationship with representatives of the most common MRSA pulsotypes circulating in South America (e.g. Chilean and USA300 Latin American variant) was detected by PFGE. Additionally, we were not able to detect the predominance of any country-specific pulsotype, suggesting a genetically heterogeneous population of MSSA isolates exhibiting the cefazolin InE in South America. Furthermore, we performed MLST analysis in representative isolates of the most prevalent pulsotypes from blood and osteomyelitis with the highest cefazolin MICs in each country. MLST typing revealed that the majority of MSSA tested (70%, $n=7$) belonged to ST5, ST8, ST30 and ST45. Our MLST data confirm the results of PFGE, indicating that MSSA isolates exhibiting the InE in South America harbour a variety of genetic backgrounds without the predominance of a single clone or clonal cluster.

β-*Lactamase typing*

We performed sequencing of the *blaZ* gene region that encodes residues 128–216 in order to determine the type of β-lactamase present in MSSA isolates from South America. This portion of the *blaZ* gene has been used previously to classify the staphylococcal penicillinase.[21] We used the same criteria indicated above for MLST in order to select isolates for typing. Among 29 isolates that were selected for β-lactamase typing, we found that the most prevalent type of β-lactamase was type A (66%), followed by type C (31%) and D (3%). Type A β-lactamase was overwhelmingly the most prevalent enzyme in bloodstream and osteomyelitis infections (67% and 63%, respectively), followed by type C (28.5% and 37.5% of isolates, respectively). Table 3 shows the cefazolin MIC distribution by type of β-lactamase enzyme. Isolates harbouring type A β-lactamase had higher mean MIC values at high inoculum than those carrying type C β-lactamase ($P=0.013$).

Discussion

Cefazolin is widely used in the treatment of *S. aureus* infections and is administered routinely as antimicrobial prophylaxis in surgery.[2,3] Cefazolin is commonly prescribed in deep-seated *S. aureus* infections when transitioning to outpatient therapy in patients who receive an initial course of isoxazolyl penicillins (which need to be administered at short intervals due to their short half-life). Cefazolin, with a longer half-life (1.8 h), is usually administered at 8 h intervals, which is more convenient for outpatient administration. Lee *et al*.[22] demonstrated that cefazolin exhibited clinical outcomes similar to nafcillin for the treatment of MSSA bacteraemia and it was better tolerated (fewer side effects) than nafcillin in a retrospective, propensity score-matched, case–control study performed in Korea. Moreover, in a retrospective cohort study by Schweizer *et al*.[23] that included patients with MSSA bacteraemia, administration of cefazolin was independently associated with a lower adjusted rate of mortality (adjusted hazard ratio: 0.21; 95% CI: 0.09–0.47) compared with vancomycin. Similarly, Stryjewski *et al*.[24] compared the outcomes of 123 patients on haemodialysis who received vancomycin or cefazolin. Treatment failure was more common in patients receiving vancomycin (31.2%) than cefazolin (13%; $P=0.02$). When the authors performed a multivariable analysis, independent factors associated with therapeutic failure included the use of vancomycin and retention of the intravenous access.[24]

Despite the clinical efficacy and good *in vitro* activity of cefazolin against MSSA, failures in the treatment of endocarditis have been documented since 1973.[25] Indeed, Quinn *et al*.[25] were the first to demonstrate that the MIC of cefazolin increased >10-fold when tested at high inoculum (10^7cfu/mL) against an MSSA isolate recovered from a patient who failed cefazolin therapy for endocarditis. Subsequently, cefazolin therapeutic failures and relapses in the treatment of MSSA strains that demonstrate the cefazolin InE have been confirmed.[4,26] Nonetheless, the overall clinical impact of the cefazolin InE in the treatment of MSSA infections has not been systematically investigated and only two previous studies[11,14] have attempted to identify the prevalence of the cefazolin InE among MSSA isolates. The first one[11] included 98 isolates recovered in several countries throughout the world from patients with a variety of *S. aureus* infections (complicated skin and soft tissue infections, hospital-acquired pneumonia, endocarditis and bacteraemia). In that study,[11] the overall prevalence of the cefazolin InE (MICs 16 to ≥128 mg/L) was only 19% (19 out of 98 isolates). In a more recent study by Livorsi *et al*.[14] that included isolates from bloodstream infections recovered from five hospitals affiliated with the same university in the USA, the cefazolin InE was found in 27% of MSSA isolates (MICs 1–32 mg/L, with a definition of InE as a ≥4-fold increase in the cefazolin MIC at high inoculum).[14] In South America, the prevalence of the InE effect was unknown, despite the fact that it may have an important clinical impact, since, at least in Colombia, cefazolin is often used as first-line therapy for severe infections caused by MSSA due to the possibility that generic oxacillin derivatives may lack *in vivo* therapeutic equivalence with that of the innovator molecule;[15] however, clinical data supporting this approach are lacking. In other countries such as Argentina, oxacillin and derivatives are not even available in the market, making cefazolin the 'drug of choice' for MSSA infections. It is important to note that a range of high-inoculum cefazolin MICs were observed among MSSA, suggesting that isolates with higher MICs may be more prone to therapeutic failure, although this correlation has not been tested clinically.

Although our data cannot be generalized to all hospitals in the region, our results are the first to show that MSSA recovered from invasive infections (bloodstream and osteomyelitis) in South American hospitals exhibit a higher frequency of the cefazolin InE than previously reported in other parts of the world. Most worrisome is the finding that almost half of MSSA isolates recovered from osteomyelitis infections (a disease that requires appropriate bactericidal therapy) exhibited such an InE. These findings may have implications for the use of cefazolin for osteoarticular

Table 3. Cefazolin MICs at high inoculum among types A and C representatives of MSSA isolates

	Susceptibility values (mg/L)				
Type of β-lactamase	mean MIC	IQR	MIC_{50}	MIC_{90}	range
Type A isolates ($n=19$)	164.21[a]	32–256	64	512	16–512
Type C Isolates ($n=9$)	39.11	16–32	32	128	16–128

[a]$P=0.0129$ calculated by Wilcoxon rank-sum test to compare mean MICs at high inoculum between isolates with type A β-lactamase versus type C.

infections in the region. Additionally, a high prevalence of InE in MSSA isolates recovered from bloodstream infections was found in Ecuador and Colombia (40% and 31%, respectively), suggesting that the activity of cefazolin may be compromised in at least one-third of patients presenting with MSSA bacteraemia in those countries and emphasizing the need for clinical data regarding the outcomes of patients treated with cefazolin in the region. Of note, the InE may also affect other cephalosporins and testing of this phenomenon with other compounds of the same class would also be of importance.

The clinical relevance of our findings is unclear. Although clinical failures when using cefazolin in high-inoculum infections such as infective endocarditis have been documented,[4,12,25,26] the paucity of clinical data precludes making strong recommendations at this point. It is also unclear if the cefazolin InE would have relevance in other infections with lower bacterial inocula. Our data suggest that in clinical settings with a high prevalence of MSSA isolates exhibiting the InE, it would be prudent for the laboratory to test for this phenotype in deep-seated MSSA infections, although the cost–benefit ratio of this approach needs to be carefully evaluated. Moreover, strategies to overcome the cefazolin InE (e.g. higher cefazolin dose or combinations with β-lactamase inhibitors) in serious MSSA infections would need to be considered if an important clinical impact of this phenotype is confirmed. Therefore, prospective clinical data are urgently needed to address this issue.

In agreement with previous reports,[11,14,27] type A was the most frequent β-lactamase detected in MSSA isolates exhibiting the cefazolin InE. This is not surprising, since type A β-lactamase has a higher kinetic affinity for cefazolin than other types.[5] Nonetheless, 31% of MSSA isolates exhibiting the InE harboured type C β-lactamase, which has also been previously associated with the cefazolin InE.[11] It is important to note that both type A and C β-lactamases are often encoded by genes present on transmissible elements, such as plasmids and transposons,[28,29] suggesting that the high prevalence of InE observed in the region may be due to the dissemination of mobile elements among MSSA, although other factors, such as hyperproduction of the enzyme,[30] may also contribute to the InE. Another important conclusion from our results is that the cefazolin InE could not be explained by the dissemination of a specific clone, since a high degree of genetic diversity was found among the MSSA exhibiting the cefazolin InE, with a prevalence of four genetic lineages that belong to the most important *S. aureus* (including MRSA) clonal complexes (CCs) disseminated worldwide (CCs 5, 8, 45 and 30).[31] Our results also suggest that similar genetic backgrounds are present in both MSSA and MRSA[17] from South America, raising the possibility that the changing molecular epidemiology of hospital-associated (HA)-MRSA in the region that has been previously reported[17,32] may originate from a pool of genetic lineages of circulating MSSA. Indeed, the two current most successful HA-MRSA clones prevalent in South America belong to CC5 (Chilean clone) and CC8 (USA300 Latin American variant), suggesting that successful MSSA lineages within CC5 and CC8 that acquired the SCC*mec* by horizontal dissemination[33–35] may explain the dynamics of the population genetics of MRSA in the region.

In summary, a high prevalence of cefazolin InE was detected among MSSA isolates from northern South America and was associated with β-lactamase types A and C, suggesting that treatment of deep-seated infections with cefazolin (particularly in Colombia and Ecuador) may be compromised. Further studies are needed to determine the clinical impact of the cefazolin InE in severe *S. aureus* infections in the region.

Acknowledgements

Part of this work was presented at the 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC 2012), San Francisco, CA, USA, 12 September 2012 (abstract number C2-1389).

Funding

This work was supported by a research award from the Universidad El Bosque, 'Research and Development Projects Program' Project PCI 2011-235. C. A. A. is supported by NIH grant R01-AI093749 from the National Institute of Allergy and Infectious Diseases (NIAID), part of the US National Institutes of Health.

Transparency declarations

None to declare.

References

1 Klevens RM, Morrison MA, Nadle J *et al*. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* 2007; **298**: 1763–71.

2 Bamberger DM, Boyd SE. Management of *Staphylococcus aureus* infections. *Am Fam Physician* 2005; **72**: 2474–81.

3 Sabath LD. Reappraisal of the antistaphylococcal activities of first-generation (narrow-spectrum) and second-generation (expanded-spectrum) cephalosporins. *Antimicrob Agents Chemother* 1989; **33**: 407–11.

4 Nannini EC, Singh KV, Murray BE. Relapse of type A β-lactamase-producing *Staphylococcus aureus* native valve endocarditis during cefazolin therapy: revisiting the issue. *Clin Infect Dis* 2003; **37**: 1194–8.

5 Zygmunt DJ, Stratton CW, Kernodle DS. Characterization of four β-lactamases produced by *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1992; **36**: 440–5.

6 Kernodle DS, Stratton CW, McMurray LW *et al*. Differentiation of β-lactamase variants of *Staphylococcus aureus* by substrate hydrolysis profiles. *J Infect Dis* 1989; **159**: 103–8.

7 Dyke KDH. β-Lactamases of *Staphylococcus aureus*. In: Hamilton-Miller JMT, Smith JT, eds. β-*Lactamases*. London: Academic Press, 1979; 291–310.

8 Soriano F, García-Corbeira P, Ponte C *et al*. Correlation of pharmacodynamic parameters of five β-lactam antibiotics with therapeutic efficacies in an animal model. *Antimicrob Agents Chemother* 1996; **40**: 2686–90.

9 Brook I. Inoculum effect. *Rev Infect Dis* 1989; **11**: 361–8.

10 Medeiros AA. Evolution and dissemination of β-lactamases accelerated by generations of β-lactam antibiotics. *Clin Infect Dis* 1997; **24** Suppl 1: S19–45.

11 Nannini EC, Stryjewski ME, Singh KV *et al*. Inoculum effect with cefazolin among clinical isolates of methicillin-susceptible *Staphylococcus aureus*: frequency and possible cause of cefazolin treatment failure. *Antimicrob Agents Chemother* 2009; **53**: 3437–41.

12 Fernández-Guerrero ML, de Górgolas M. Cefazolin therapy for *Staphylococcus aureus* bacteremia. *Clin Infect Dis* 2005; **41**: 127.

13 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-first Informational Supplement M100-S21*. CLSI, Wayne, PA, USA, 2012.

14 Livorsi DJ, Crispell E, Satola SW *et al*. Prevalence of *blaZ* gene types and the inoculum effect with cefazolin among bloodstream isolates of methicillin-susceptible *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2012; **56**: 4474–7.

15 Rodriguez CA, Agudelo M, Zuluaga AF *et al*. In vitro and in vivo comparison of the anti-staphylococcal efficacy of generic products and the innovator of oxacillin. *BMC Infect Dis* 2010; **10**: 153.

16 Arias CA, Reyes J, Zúñiga M *et al*. Multicentre surveillance of antimicrobial resistance in enterococci and staphylococci from Colombian hospitals, 2001–2002. *J Antimicrob Chemother* 2003; **51**: 59–68.

17 Reyes J, Rincón S, Díaz L *et al*. Dissemination of methicillin-resistant *Staphylococcus aureus* USA300 sequence type 8 lineage in Latin America. *Clin Infect Dis* 2009; **15**: 1861–7.

18 Martineau F, Picard FJ, Lansac N *et al*. Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob Agents and Chemother* 2000; **44**: 231–8.

19 Murray BE, Singh KV, Heath JD *et al*. Comparison of genomic DNAs of different enterococcal isolates using restriction endonucleases with infrequent recognition sites. *J Clin Microbiol* 1990; **28**: 2059–63.

20 Enright MC, Day NP, Davies CE *et al*. Multilocus sequence typing for characterization of meticillin-resistant and meticillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000; **38**: 1008–15.

21 Voladri RK, Kernodle DS. Characterization of a chromosomal gene encoding type B β-lactamase in phage group II isolates of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1998; **42**: 3163–8.

22 Lee S, Choe PG, Song KH *et al*. Is cefazolin inferior to nafcillin for treatment of methicillin-susceptible *Staphylococcus aureus* bacteremia? *Antimicrob Agents Chemother* 2011; **55**: 5122–6.

23 Schweizer ML, Furuno JP, Harris AD *et al*. Comparative effectiveness of nafcillin or cefazolin versus vancomycin in methicillin-susceptible *Staphylococcus aureus* bacteremia. *BMC Infect Dis* 2011; **11**: 279.

24 Stryjewski ME, Szczech LA, Benjamin DK Jr. Use of vancomycin or first-generation cephalosporins for the treatment of hemodialysis-dependent patients with methicillin-susceptible *Staphylococcus aureus* bacteremia. *Clin Infect Dis* 2007; **44**: 190–6.

25 Quinn EL, Pohlod D, Madhavan T *et al*. Clinical experiences with cefazolin and other cephalosporins in bacterial endocarditis. *J Infect Dis* 1973; **128** Suppl: S386–9.

26 Bryant RE, Alford RH. Unsuccessful treatment of staphylococcal endocarditis with cefazolin. *JAMA* 1977; **237**: 569–70.

27 Shuford JA, Piper KE, Hein M *et al*. Lack of association of *Staphylococcus aureus* type A β-lactamase with cefazolin combined with antimicrobial spacer placement prosthetic joint infection treatment failure. *Diagn Microbiol Infect Dis* 2006; **54**: 189–92.

28 East AK, Dyke KG. Cloning and sequence determination of six *Staphylococcus aureus* β-lactamases and their expression in *Escherichia coli* and *Staphylococcus aureus*. *J Gen Microbiol* 1989; **135**: 1001–15.

29 Gillespie MT, Lyon BR, Skurray RA. Structural and evolutionary relationships of β-lactamase transposons from *Staphylococcus aureus*. *J Gen Microbiol* 1988; **134**: 2857–66.

30 Barg N, Chambers H, Kernodle D. Borderline susceptibility to antistaphylococcal penicillins is not conferred exclusively by the hyperproduction of β-lactamase. *Antimicrob Agents Chemother* 1991; **35**: 1975–9.

31 Monecke S, Coombs G, Shore AC *et al*. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. *PLoS One* 2011; **6**: e17936.

32 Cruz C, Moreno J, Renzoni A *et al*. Tracking methicillin-resistant *Staphylococcus aureus* clones in Colombian hospitals over 7 years (1996–2003): emergence of a new dominant clone. *Int J Antimicrob Agents* 2005; **26**: 457–62.

33 Rolo J, Miragaia M, Turlej-Rogacka A *et al*. High genetic diversity among community-associated *Staphylococcus aureus* in Europe: results from a multicenter study. *PLoS One* 2012; **7**: e34768.

34 Vivoni AM, Diep BA, de Gouveia Magalhães AC *et al*. Clonal composition of *Staphylococcus aureus* isolates at a Brazilian university hospital: identification of international circulating lineages. *J Clin Microbiol* 2006; **44**: 1686–91.

35 Enright MC, Robinson DA, Randle G *et al*. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proc Natl Acad Sci USA* 2002; **99**: 7687–92.