cluster was typed by MLST according to the Pasteur Institute scheme (www.pasteur.fr/mlst), giving rise to the subsequent clones ST326 (CX1), ST405 (CX2), ST147 (CX3), ST104 (CX4 and CX5) and ST15 (CX6) (Figure 1). Of note, all clones except ST104 had previously been associated with OXA-48 production in Europe and/or Spain.[1,8,9]

The concomitant occurrence of five different *K. pneumoniae* clones producing OXA-48 in a single hospital might be explained, at least in part, by the high transfer frequency of the IncL/M plasmid carrying the bla_{OXA-48} gene.[10] In view of our data, it is likely that the spread of OXA-48-producing *K. pneumoniae* in Spain will rapidly mirror the endemic situation observed in Italy and the USA with KPC-producing *K. pneumoniae* isolates. Once again, this bacterial species is playing a pivotal role in the emergence and dispersion of resistance traits in hospital settings. Urgent hygiene measures have to be taken to prevent further consolidation of a difficult-to-control situation.

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Transparency declarations

None to declare.

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In vivo emergence of ceftaroline resistance during therapy for MRSA vertebral osteomyelitis

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Sir,

Ceftaroline, the active metabolite of ceftaroline fosamil, was approved by the US FDA in 2010 and by the European Commission in 2012 for the treatment of acute bacterial skin and skin structure infections and community-acquired bacterial pneumonia.[1] This medication has also been reported to be used for the sporadic treatment of other severe MRSA infections, including osteomyelitis[2] and epidural abscesses.[3]

A middle-aged man with diabetes mellitus was admitted for incision and drainage of a septic left wrist. Source control was achieved. Tissue cultures grew MRSA (strain 89, Table 1), with ceftaroline MIC of 0.75 mg/L by broth microdilution (incremental dilution steps).[4] Vancomycin was initiated, but was switched to ceftaroline fosamil due to rising serum creatinine and impending cardiac catheterization for a myocardial infarction. He improved and was discharged on renally adjusted ceftaroline fosamil 600 mg every 12 h. During his hospitalization, the patient complained of chronic sciatica back pain. Physical examination was notable for no spinal tenderness. However, back pain progressed post-discharge, and MRI showed vertebral osteomyelitis and collections in bilateral psoas muscles. He was readmitted for CT-guided aspiration of the right psoas fluid collection, which grew MRSA (strain 91) with ceftaroline MIC 4–6 mg/L (Table 1) and antibiotics were changed to vancomycin. Peripheral blood cultures revealed two MRSA isolate morphologies (strains 86 and 88). He underwent laminectomy and facetectomy for epidural abscess drainage with lumbar debridement, fusion and fixation. Cultures from the epidural abscess again grew MRSA. Trans-thoracic echocardiogram was negative for vegetations. The patient was discharged and completed 12 weeks of intravenous antibiotics followed by 12 months of oral doxycycline after inflammatory markers normalized. More than 1 year after surgery the patient was ambulating independently with a cane and living at home, and inflammatory markers had remained normal for >4 months after completing antibiotics. The patient gave verbal and written informed consent for the publication of this case report.

The baseline MRSA (strain 89) and subsequent ceftaroline-resistant isolates (strains 86, 88 and 91) were subjected to WGS. Genome sequence data were utilized to extract epidemiological information related to isolate ST, *spa* and SCC*mec* determination, as previously described.[5] The DNA genes and/or

Table 1. Series of MRSA isolates included in the study

Isolate	Specimen	Collection date	Time frame (days/weeks)[a]	Genome comparison[b]			Ceftaroline MIC (mg/L)	PBP2a amino acid residue number			Clonal type[c]
				SNPs	InDels	total		nPBD	PBD 447	PBD 601	
89	wound (wrist)	1/2/14	NA	NA	NA	NA	0.75/0.75/0.75	WT	Glu	Ala	ST5-II
91	wound (lumbar spine)	1/3/14	28/4	104	70	174	4/4/6	WT	Lys	Ser	ST5-II
86	blood (colony 1)	13/3/14	40/6	93	64	157	8/8/8	WT	Lys	Ser	ST5-II
88	blood (colony 2)	13/3/14	40/6	92	59	151	4/6/6	WT	Lys	Ser	ST5-II

nPBD, non-penicillin-binding domain.
[a]Period after recovery of baseline MRSA isolate.
[b]Numbers of SNPs, insertions and deletions (InDels) compared with baseline genome.
[c]Combination of MLST (ST) type and SCC*mec* type. All isolates were *spa* type t002/TJMBMDMGMK (Ridom/Kreiswirth).

regions obtained from the baseline MRSA (strain 89) were considered as the reference for comparison purposes with the three selected follow-up isolates included in the study. The following proteins were investigated: PBP1, PBP2, PBP2a, PBP3 and PBP4. Other DNA sequence regions analysed were *mecI*, *mecR* and the *mecA* ribosomal binding site and the *vraSR* regulation network (cell wall stress response operon). All downstream DNA analysis was performed using the Lasergene® package (DNAStar).

All four isolates belonged to ST5, had a *spa* type t002 and carried an SCC*mec* type II, associated with MRSA USA100 clone.[6] Follow-up isolates showed Glu447Lys and Ala601Ser alterations in the penicillin-binding domain (PBD) of PBP2a. The additional alteration observed at the PBD (Ala601Ser) has not been previously reported and the role of this mutation in the phenotype associated with decreased susceptibility to ceftaroline needs further investigation. However, the adjacent amino acid (Glu602) is involved in a conformational change caused by the ceftaroline R1 segment, which is associated with a broader conformational change linked to opening the active site.[7] Thus, it can be speculated that the Ala601Ser mutation may be associated with decreased ceftaroline binding to the active site. In addition, the Glu447 amino acid is adjacent to the critical Tyr446 in the cephalosporin binding pocket, which closely interacts with the R2 group of ceftaroline and ceftobiprole.[6,7] Therefore, the resistant strains described here may have possessed two mutations affecting binding at both the R1 and R2 ceftaroline groups. The other PBP amino acid sequences and other DNA sequence regions investigated were unchanged in the three follow-up isolates compared with the reference strain 89. In summary, clinical failure with ceftaroline may indicate emerging resistance. The role of this newly described resistance mutation in MRSA needs further investigation.

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Steady-state pharmacokinetics of oral linezolid suspension in a premature infant with osteomyelitis

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Sir,

Linezolid has activity against a broad range of Gram-positive bacteria, including MRSA. As infections with these organisms have become more common in infants, linezolid may have an important role, particularly in preterm newborns. Most of the published data on pharmacokinetics, efficacy and safety of linezolid are from adult studies.[1,2] It is established that the pharmacokinetics of linezolid, especially clearance, varies with age. Children younger than 12 years of age have a smaller AUC, faster clearance and shorter elimination half-life than adults. Paediatric data, including from neonates, are limited and were mainly evaluated using the intravenous (iv) formulation.[2–6] These studies reported considerable interindividual variability in plasma concentrations within the study populations. To date, there are no steady-state pharmacokinetic studies assessing oral linezolid suspension in infants. We report the steady-state pharmacokinetic parameters of oral linezolid suspension in a premature infant with osteomyelitis. The project was approved by the institutional review board, informed consent was obtained and the research was conducted in accordance with the Declaration of Helsinki.

A 4-month-old male infant born at 25 weeks gestation (birth weight 750 g) was being managed in the neonatal ICU for ongoing medical issues since birth; iv access proved challenging throughout the hospital course. On the 44th day of life, the infant developed a skin and soft-tissue infection of the left forearm at an old iv site, along with spontaneously draining pustules at the umbilicus. Cultures obtained from the forearm abscess grew MRSA with a vancomycin MIC of 2 mg/L. The isolate also was resistant to clindamycin. The infant was treated with vancomycin iv for 10 days, although a therapeutic trough was not achieved (3.6 mg/L). On the 89th day of life, the infant developed left ankle swelling at another old iv access site. Imaging revealed significant osteomyelitis of the distal tibia and fibula. Cultures from the bone grew a latex-negative *Staphylococcus*. However, further speciation and susceptibilities were not conducted as the laboratory discarded the sample. The infant was started on linezolid, iv at 10 mg/kg (29 mg) q8h. Treatment was monitored with serial serum inflammatory markers. Parenteral therapy was disrupted on several occasions as iv access was compromised. After 14 days of parenteral therapy, the infant was switched to oral linezolid suspension, 10 mg/kg q8h, for an additional 4 weeks. Blood samples obtained during the last week of oral linezolid therapy were analysed for quantification of linezolid using HPLC. Blood samples (0.5 mL) were obtained at time 0 before dosing and at 1, 1.5, 2, 4, 6 and 8 h after the oral dose of linezolid. An additional sample, 10 h after the prior dose and 2 h after the afternoon dose, also was obtained. Serum samples were shipped to the Infectious Disease Pharmacokinetics Laboratory in Gainesville (FL, USA) for serum concentration analysis. Linezolid concentrations were determined using a validated HPLC assay described previously.[7]

The plasma standard curve for linezolid ranged from 0.50 to 30 mg/L, with linearity extending below 0.50. The within-sample precision (percentage coefficient of variation) of validation in a single standard concentration was 0.69% and the overall validation precision across all standards was 1.04%–4.39%. Non-compartmental analysis of the data was performed with Phoenix software (v.6.4, Pharsight) to obtain the steady-state pharmacokinetic parameters. The 0 and 8 h sample concentrations (minimum plasma concentration, C_{min}) were both 0.32 mg/L, confirming that steady-state had been achieved (Figure 1). The steady-state peak plasma concentration (C_{max}) was 5.51 mg/L and the time to peak plasma concentration (T_{max}) was 2 h. The second 2 h sample (10 h after the prior oral dose) was 3.99 mg/L, somewhat lower than the prior C_{max} of 5.51 mg/L. This suggests

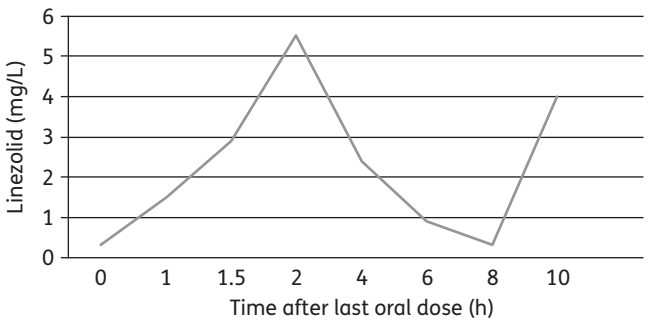

Figure 1. Steady-state linezolid plasma concentrations.