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Characterization of Methicillin-resistant *Staphylococcus aureus* Bloodstream Isolates Recovered from Patients Enrolled in a Randomized, Double-blind, Multi-center Study to Establish the Efficacy and Safety of Ceftobiprole for Treatment of Bacteremia, Including Infective Endocarditis

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Introduction

- Ceftobiprole is an advanced-generation cephalosporin that has *in vitro* and *in vivo* activity against clinically important Gram-positive organisms, including MRSA, and Gram-negative organisms.
- Ceftobiprole was approved in Europe and many non-European countries for the treatment of community-acquired bacterial pneumonia (CABP) and non–ventilator-associated hospital-acquired bacterial pneumonia in adults caused by indicated species.
- A recent Phase 3 clinical trial evaluated ceftobiprole for the treatment of *Staphylococcus aureus* bacteremia, including right-sided infective endocarditis (NCT03138733).
- A New Drug Application for ceftobiprole was recently submitted to the United States (US) Food and Drug Administration seeking its approval for *Staphylococcus aureus* bacteremia, including right-sided infective endocarditis, acute bacterial skin and skin structure infections, and CABP.
- This study reports the molecular characteristics of MRSA isolates recovered from patients enrolled in a phase 3 clinical trial for ceftobiprole and the clinical outcomes associated with different genotypes.

Materials and Methods

Bacterial Isolates

- A total of 94 patients had MRSA isolated from baseline blood cultures, 90 of which were available for molecular characterization and included in the study. Two patients didn't have *S. aureus* recovered during the screening visits available for testing, so isolates recovered during Day 1 for these 2 patients were included in this analysis.

Susceptibility testing

- Isolates were tested for antimicrobial susceptibility using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method.
- Quality assurance was performed by sterility checks, colony counts, and testing CLSI-recommended quality control reference strains.

Molecular Analysis

- Total genomic DNA was extracted and purified using the KingFisher Cell and Tissue DNA kit (Thermo Scientific, Waltham, Massachusetts, USA) in a robotic KingFisher™ Flex Magnetic Particle Processor (Thermo Scientific) workstation.
- Total genomic DNA was used as input material for library construction. DNA libraries were prepared using the Nextera XT™ library construction protocol and index kit (Illumina, San Diego, California, USA) and sequenced on a MiSeq Sequencer (Illumina) using a MiSeq Reagent Kit v3 (600 cycle). Sequencing reactions were initially performed to achieve DNA read lengths of up to 300-bp and an average genome coverage depth of approximately 30x.
- Each raw data set was quality assured, error corrected, and assembled *de novo* using assembler SPAdes 3.11.1. Assembled genomes had the DNA sequence information extracted to determine the MLST as well as *spa*, SCC*mec* typing and other genetic information among MRSA strains. A clonal type designation (e.g., USA100) was assigned to the baseline *S. aureus* isolates when possible based on the molecular information obtained.

Results

- A total of 10 clonal complexes (CC) were present within the 90 isolates, and 71 (78.9%) MRSA were grouped into 3 predominant clones: CC8, CC22, and CC5 (Table 1).
- Among CC8 (37/90; 41.1%), 4 ST8-like (4/37; 10.8%) from USA (3) and Ukraine (1) were observed. Isolates from the USA carried SCC*mec* IVa, PVL and ACME, and were designated as USA300.
  - Additional CC8 isolates included 12 ST239 (12/37; 32.4%) from Bulgaria (1), Georgia (1), Russia (1), Serbia (2), and Ukraine (7) were designated as the Hungarian clone.
  - Other ST8-like MRSA (19/37; 51.4%) from Ukraine (15), Russia (2), Spain (1), and Argentina (1) were identified as USA300LAV, differentiated from USA300 by the presence of SCC*mec* IVc.
- Nineteen (19/90; 21.1%) MRSA belonged to CC22/ST22.
- Fifteen (15/90; 16.7%) MRSA clustered within CC5. These isolates included ST5 and related single- (ST225 and ST2704) or double-locus (ST1827) variants and were designated as USA100 (7/15; 46.7%) or USA800 (8/15; 43.3%).
- Other less prevalent lineages include:
  - Five (5.6%) isolates recovered from patients hospitalized in Ukraine that belonged to CC59/ST59, had *spa* type t437/t4911, and carried SCC*mec* type V (5C2&5).
  - Four (4.4%) MRSA belonging to CC1 (ST1; t127, t177) from Bulgaria (2), Russia (1), and Ukraine (1) or CC398 (ST398; t034, t108, t779, and t898) recovered from Bulgaria (1), Georgia (1), and Ukraine (2).
  - The remaining MRSA strains investigated belonged to CC45/ST45 (2/90; 2.2%) and carried SCC*mec* type IV (Berlin clone) or V (unknown clone); CC30 (2/90; 2.2%) comprised with 2 strains associated with ST30/ST1829 and SCC*mec* type IV (USA1100); and 1 (1.1%) strain each of CC152/ST152 and CC97/ST1153.
- Overall, clinical responses and microbiological eradication were similar between treatment arms (Table 2).
  - Clinical response and microbiological eradication rates were higher in the ceftobiprole arm among patients infected with CC5 strains compared to daptomycin.
  - Clinical response and microbiological eradication rates were higher in the daptomycin arm among patients infected with CC22 and other CC types compared to ceftobiprole.

Conclusions

- This global *S. aureus* bacteremia clinical trial for ceftobiprole had patients infected with MRSA strains, most of which belonged to the main pandemic lineages CC8, CC22, and CC5.
- Important strains were identified within CC8:
  - All CC8 strains from the USA belonged to the USA300 clone, which reflects the predominance of these strains causing invasive infections in the USA.
  - The Latin American variant of USA300 (USA300LAV) was the most prevalent (19/37; 51.4%) strain within CC8. This strain initially evolved in Latin America and was contained within this region; however, these strains seem to have spread in many countries outside Latin America.
  - ST239-MRSA-III (12/37; 32.4%) was the second most common strain within CC8, and represents one of the oldest pandemic MRSA strains, displays a multidrug resistance (MDR) susceptibility profile, and remains highly prevalent among nosocomial strains in certain regions of the globe, especially countries in the Asia-Pacific region.
- CC22 (19/90; 21.1%) and CC5 (15/90; 16.7%) were the second and third most common MRSA lineages detected, respectively, which reflects their widespread dissemination.
- Other MRSA strains represented less prevalent clonal types (each <6%).
  - Interestingly, 4 strains collected from 3 European countries belonged to ST398-MRSA, which has been extensively described as a pathogen in livestock animals over the last two decades, and a causative agent of diseases in humans.
  - More recently, ST1-MRSA-V also emerged as a common strain in livestock animals in Italy.
- Overall, this clinical strain set includes clones previously associated with hospital- and community-acquired infections as well as strains associated with livestock infections.
  - The MRSA lineages identified here represents those causing infections in patients hospitalized in United States medical centers.
- In general, clinical responses and microbiological eradication were comparable between the 2 study arms with small differences in outcomes by CC type. However, the number of patients within each CC type was small, limiting conclusions.

Disclosures

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Table 1. Epidemiologic information obtained for baseline MRSA strains

Number	CC[a]	MLST	*spa*	SCC*mec*[b]	Clone[c]	BPR MIC (mg/L)[d]	Country
19	CC8	8	t008/t024/t051/t190/t574	IVc (2B)	USA300LAV	1	UKR (15); RUS (2), ESP (1); ARG (1)
2		8	t008	IVa (2B)	USA300		USA
1		8-like	t622	IVa (2B)	USA300		USA
1		8-like	t008	IVa (2B)	USA500		UKR
1		72	t1597	IVa (2B)	USA700		RUS
12		239	t030/t037/t631/t748/t3519	III (3A)	Hungarian	2	BGR (1); GEO (1), RUS (1); SRB (2); UKR (7)
1		247	t052	I (1B)	Iberian	2	UKR
12	CC22	22	t005/t309	IVa (2B)	UK-EMRSA-15	1	UKR
3			t032/t379/t608	IVj (2B)	UK-EMRSA-15		ISR
2			t032/t608	IV (2B)	UK-EMRSA-15		ISR
1			t12437	IVc (2B)	UK-EMRSA-15		RUS
1			t032	IVh (2B)	UK-EMRSA-15		RUS
3	CC5	5	t002/t895	II (2A)	USA100	2	ISR (2); MEX (1)
3		1827	t003	II (2A)	USA100		UKR
1		225	t003	II (2A)	USA100		GRC
1		5	t002	IVa (2B)	USA800	1	ARG
1			t1154	IVb (2B)	USA800		COL
3			t002/t14303	IVc (2B)	USA800		RUS (2); UKR (1)
2			t306	IVi (2B)	USA800		UKR
1		2704	t002	IVc (2B)	USA800		UKR
5	CC59	59	t437/t4911	V (5C2&5)	Taiwan	1	UKR
3	CC1	1	t127/t177	IVa (2B)	USA400	1	BGR (2); RUS (1)
1			t127	V (5C2)	ST1-LA-MRSA		UKR
3	CC398	398	t034/t779/t898	V (5C2&5)	ST398-LA-MRSA	0.5	BGR (1); UKR (2)
1			t108	IVa (2B)	ST398-LA-MRSA	1	GEO
1	CC45	45	t015	IVa (2B)	Berlin	0.5-1	BGR
1			t1081	V (5C2&5)	Unknown		USA
1	CC30	30	t019	IVc (2B)	USA1100	1	ISR
1		1829	t1143	IVa (2B)	USA1100		BGR
1	CC152	152	t4019	NT	Unknown	0.5	UKR
1	CC97	1153	t903	NT	Unknown	0.5	UKR

CC, clonal complex; MLST, multilocus sequence typing; SCC*mec*, staphylococcal cassette chromosome; BPR, ceftobiprole; NT, non-typeable; USA300LAV, Latin American variant; LA, livestock-associated; ARG, Argentina; BGR, Bulgaria; COL, Colombia; ESP, Spain; GEO, Georgia; GRC, Greece; ISR, Israel; MEX, Mexico; RUS, Russia; SBR, Serbia; UKR, Ukraine; USA, United States.
[a] CC assigned based on MLST through eBurst analysis.
[b] SCC*mec* type and subtype. Numbers and letters between parentheses represent *ccr* complexes and SCC*mec* gene complexes, respectively.
[c] At least 1 presumptive clonal strain designation based on ST, SCC*mec*, *spa* typing and other genetic characteristics, such as PVL and ACME.
[d] Modal MIC described for CC types, unless the CC was represented by a single isolate. CC8, CC5, and CC398 are represented by SCC*mec* type.

Table 2. Clinical response and microbiological eradication among patients infected with MRSA according to genotype

MRSA Clonal Complex	Ceftobiprole			Daptomycin		
	No. of MRSA patients	% Clinical Response[a]	% Microbiological Eradication[a]	No. of MRSA patients	% Clinical Response[a]	% Microbiological Eradication[a]
All	43	69.0	81.4	47	78.7	83.0
CC8	18	72.2	83.3	19	78.9	78.9
CC22	8	62.5	75.0	11	90.9	100
CC5	8	87.5	100	7	57.1	57.1
Other[b]	9	55.5	66.7	10	80.0	90.0

[a] Positive clinical response in the modified intent-to-treat (mITT) population was assessed at post-treatment evaluation (Day 70). Microbiological eradication was defined as a negative blood culture for *S. aureus* and confirmed by a second negative blood culture obtained at least 24 hours after the first negative blood culture. Clinical response and microbiological eradication required the patient to be blood culture negative at the post-treatment evaluation visit.
[b] Includes CC59/ST59 (5/90; 5.6%), CC1/ST1 (4/90; 4.4%), CC398/ST398 (4/90; 4.4%), CC45/ST45 (2/90; 2.2%), CC30/ST30-ST1829 (2/90; 2.2%), and 1 (1/90; 1.1%) strain each of CC152/ST152 and CC97/ST1153.

References

Grundmann H, Aanensen DM, van den Wijngaard CC, Spratt BG, Harmsen D and Friedrich AW. Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. *PLoS Med.* 7: e1000215: 2010.

Hamed K, Engelhardt M, Jones ME, Saulay M, Holland TL, Seifert H, Fowler VG Jr. Ceftobiprole versus daptomycin in *Staphylococcus aureus* bacteremia: A novel protocol for a double-blind, Phase III trial. *Future Microbiol*.15: 35-48, 2020.

Merialdi G, Feltrin F, Gaetarelli B, Lombardi G, Iurescia M, Alba P, Luppi A, Martinelli N, Ramini M, Carfora V, Franco A, Battisti A. Livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) *spa* type t127, sequence type (ST) 1, quickly spreads and persists among young pigs. *Pathog Dis*. 77: ftz033: 2019.

Monecke S, Coombs G, Shore AC, Coleman DC, Akpaka P, Borg M, Chow H, Ip M, Jatzwauk L, Jonas D, Kadlec K, Kearns A, Laurent F, O'Brien FG, Pearson J, Ruppelt A, Schwarz S, Scicluna E, Slickers P, Tan HL, Weber S and Ehricht R. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus aureus*. *PLoS One* 6: e17936: 2011.

Pilla R, Castiglioni V, Gelain ME, Scanziani E, Lorenzi V, Anjum M, Piccinini R. Long-term study of MRSA ST1, t127 mastitis in a dairy cow. *Vet Rec*. 170: 312: 2012.

Turner NA, Sharma-Kuinkel BK, Maskarinec SA, Eichenberger EM, Shah PP, Carugati M, Holland TL, Fowler VG Jr. Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. *Nat Rev Microbiol*. 17: 203-218: 2019.

Holland TL, Cosgrove SE, Doernberg SB, Jenkins TC, Turner NA, Boucher HW, Pavlov O, Titov I, Kosulnykov S, Atanasov B, Poromanski I, Makhviladze M, Anderzhanova A, Stryjewski ME, Assadi Gehr M, Engelhardt M, Hamed K, Ionescu D, Jones M, Saulay M, Smart J, Seifert H, Fowler VG Jr; ERADICATE Study Group. Ceftobiprole for treatment of complicated *Staphylococcus aureus* bacteremia. *N Engl J Med*. doi: 10.1056/NEJMoa2300220. Epub ahead of print. PMID: 37754204, 2023.

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