

A Highly Virulent *Staphylococcus aureus*: Rabbit Anterior Chamber Infection, Characterization, and Genetic Analysis

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PURPOSE. To describe and characterize a *Staphylococcus aureus* strain with unique virulence that overcomes host defenses of the rabbit anterior chamber and mimics clinical cases of postcataract surgery endophthalmitis.

METHODS. Nine isolates of *S. aureus* were tested to determine their viability in the rabbit anterior chamber. Growth of UMCR1 in the anterior chamber was established and expressed as log colony-forming units per milliliter of aqueous humor. Pathologic changes produced by UMCR1 were documented by photographs, slit lamp examination, histopathologic analysis, and quantification of neutrophils. UMCR1 was characterized by antibiotic susceptibility, biochemical tests, ribotyping, genome restriction mapping, and multilocus sequence typing (MLST).

RESULTS. UMCR1 was the only *S. aureus* strain that grew within the anterior chamber, reaching $\log 6.97 \pm 0.18$ CFU/mL by 16 hours after infection. Pathologic changes included conjunctival injection, chemosis, corneal edema, severe iritis, fibrin accumulation, and a 193-fold increase in neutrophils by 16 hours after infection. UMCR1 was only resistant to sulfamethoxazole and, like other *S. aureus* isolates, polymyxin B. UMCR1 also had biochemical reactions and a ribotype pattern typical of *S. aureus*. The genomic reconstruction analysis of UMCR1 was most similar to strains MW2 and MSSA476. MLST revealed a 1 in 3198 nucleotide difference between UMCR1 and strains MW2 and MSSA476.

CONCLUSIONS. This study describes a unique *S. aureus* strain that overcomes host defenses and replicates in the anterior chamber. The survival and growth of this organism could be used for studies of *S. aureus* pathogenesis, host defenses, and effectiveness of antibiotics within the anterior chamber. (*Invest Ophthalmol Vis Sci.* 2010;51:5114–5120) DOI:10.1167/iov.10-5179

The pathogen *Staphylococcus aureus* is responsible for many human diseases, including food poisoning, soft tissue infections, pneumonia, and osteomyelitis.^{1–4} *S. au-*

reus can asymptotically colonize human skin and mucous membranes including the lid and conjunctiva of the eye.^{1,5–7} The presence of *S. aureus* on the mucous membranes and surrounding skin of the eye facilitates transmission of the organism to the anterior chamber during cataract surgery.^{5,8–14} Of all cataract surgery-related endophthalmitis cases, 70% to 80% are associated with staphylococci and approximately 10% are caused by *S. aureus*.^{15,16} If not treated promptly, endophthalmitis resulting from postcataract surgery infections with *S. aureus* can be severe and may result in loss of vision or possibly the entire eye. Treatment often becomes more problematic, and the possibility of permanent damage is more likely when endophthalmitis is caused by an antibiotic-resistant form such as methicillin-resistant *S. aureus* (MRSA).

Since *S. aureus* causes a significant portion of endophthalmitis infections, a reproducible animal model of *S. aureus* endophthalmitis in which the bacteria readily replicate within the anterior chamber is necessary for the study of bacterial pathogenesis, host defenses, and the effectiveness of treatments. Growth of *S. aureus* in the anterior chamber would necessitate inhibition or resistance of the host defenses of the anterior chamber and aqueous humor. Inflammation in the anterior chamber has been created in previous animal models by injecting bacteria into the anterior chamber.^{17–20} However, such models do not produce an active infection, and the pathologic processes of these inflammatory models are therefore inherently different from clinical infections. Girgis et al.¹⁷ have shown that the injection of *S. aureus*, even in high numbers, into the anterior chamber of rabbits results in rapid death of the bacteria due to the host defenses, some of which are poorly defined. Kowalski et al.^{18,19} have described an *S. aureus* strain that, on occasion, survived in the anterior chamber of rabbits, yet demonstrated inconsistent and limited growth. Thus, there is no model that consistently mimics a productive *S. aureus* infection in the anterior chamber similar to clinical cases of postcataract surgery endophthalmitis.

In the present study, multiple strains of *S. aureus* were tested for growth in the rabbit anterior chamber. A reproducible anterior chamber infection was established in which a unique strain of *S. aureus* (UMCR1) was found to readily replicate and cause severe disease. This study also includes genetic characterizations and comparisons of UMCR1 to other *S. aureus* isolates. The quantitatively defined bacterial growth and the progression of disease created by *S. aureus* strain UMCR1 within the anterior chamber may be used in the future to elucidate the complex pathogenesis of clinical endophthalmitis and to serve as a quantitative model for antibiotic and anti-inflammatory studies.

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MATERIALS AND METHODS

Animals

Specific pathogen-free New Zealand White rabbits were maintained according to the institutional guidelines and tenets of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Before infection or euthanatization, the rabbits were anesthetized with a 1-mL injection of 100 mg/mL xylazine (Rompum) and 100 mg/mL ketamine hydrochloride (Ketaset; Fort Dodge, Fort Dodge, IA) in a 1:5 (vol/vol; xylazine/ketamine) mixture. They were euthanatized before harvest of aqueous humor with an overdose of pentobarbital sodium (Sigma-Aldrich, St. Louis, MO) injected intravenously.

Bacterial Strains

S. aureus strains used in these experiments included 8325-4, Newman, UMCRI, MW2, and five clinical ocular isolates: 91-717, 3161-06, 30103, 177, and T1. Strains 8325-4 and Newman are laboratory strains that have been described in infections of the rabbit cornea.^{21,22}

Strain UMCRI was isolated from an ocular conjunctival infection of a rabbit delivered to the University of Mississippi Medical Center. The strain was catalase and coagulase positive and the morphology and Gram's stain reaction were consistent with *S. aureus* isolates. UMCRI colonies produced zones of β -hemolysis on sheep or rabbit blood agar. All *S. aureus* strains were grown in tryptic soy broth (TSB, BD Biosciences, Sparks, MD) at 37°C overnight. They were then supplemented with glycerol (18%), and aliquots were stored at -80°C.

Anterior Chamber Inoculation

S. aureus strains were each grown in TSB at 37°C overnight, subcultured in TSB, and grown to approximately 1×10^8 colony-forming units (CFU) per milliliter. The bacteria were then diluted in TSB and plated, in triplicate, on tryptic soy agar (TSA; BD Biosciences) to quantify the number of bacteria in the inoculum. After anesthesia, 1 drop of proparacaine hydrochloride (0.5%; Bausch & Lomb, Tampa, FL) was topically applied to each rabbit eye. The rabbits were divided into three groups, each employing a different procedure: group 1, injection with *S. aureus* (1×10^4 CFU) in 10 μ L of TSB through the cornea into the anterior chamber of each eye using a 30-gauge needle; group 2, aqueous humor was removed and the anterior chamber refilled with phosphate-buffered saline (PBS) 100 mM phosphate, and 150 mM sodium chloride [pH 7.4] followed by injection of bacteria, as described in group 1; and group 3, *S. aureus* (1×10^4 CFU) was mixed with 100 μ L of sodium hyaluronate (ProVisc; Alcon Laboratories, Inc., Fort Worth, TX) and injected into the anterior chamber after removal of the aqueous humor. The rabbit eyes were closely monitored for the development of disease, and the aqueous was harvested at 24 hours after infection (PI) for bacterial quantification. Aqueous humor from eyes inoculated with UMCRI was harvested at 16 hours PI due to the severity of the infection.

Growth Curve

Growth of *S. aureus* strain UMCRI in the anterior chamber was analyzed in more detail. Rabbits injected with 1×10^4 CFU of UMCRI in 10 μ L of TSB into the aqueous humor were killed at various time points after infection. The aqueous humor of each eye ($n \geq 4$ per time point) was collected, and each sample of aqueous humor was serially diluted in PBS. Aliquots of aqueous humor dilutions were then plated in triplicate on TSA and incubated overnight at 37°C to determine log CFU/mL of aqueous humor.

Slit Lamp Examination

Rabbit eyes ($n = 4$ per group) were evaluated for the progression of endophthalmitis via slit lamp examination (SLE) performed by two masked observers with a biomicroscope (Topcon SL-7E; Kogaku Kikai K.K., Tokyo, Japan). Seven parameters were evaluated for each eye: injection, chemosis, iritis, hypopyon, corneal infiltrate, corneal edema,

and fibrin in the anterior chamber. Each of these parameters was given a grade ranging from 0 (normal) to 4 (severe). The sum of the grades from each observer were averaged yielding a total SLE score that could range from 0 (normal eye) to a theoretical maximum of 28. Photographs were also taken over the course of infection.

Histopathologic Analysis

Whole rabbit eyes having the anterior chamber injected with 10 μ L of TSB alone ($n = 1$) or 10^4 CFU of UMCRI ($n = 2$) in TSB were randomly selected and harvested 16 hours after injection. Whole eyes were placed in a fixative solution (Excalibur Pathology, Moore, OK) and were processed by Excalibur Pathology. Samples were processed by embedding the eyes in paraffin, sectioning, mounting on slides, and staining with hematoxylin and eosin. The histologic sections were then analyzed under a microscope.

Myeloperoxidase Assay

The aqueous humor of infected rabbit eyes ($n = 4$) and eyes injected with TSB alone ($n = 4$) was assessed for the presence of polymorphonuclear leukocytes (PMNs) by a myeloperoxidase (MPO) assay 16 hours after injection. MPO assays were performed with a colorimetric *o*-dianisidine reaction, as previously described.²³⁻²⁵ One unit of MPO activity was equivalent to $\sim 2 \times 10^5$ PMNs. Assays were performed in triplicate.

Biochemical and Genetic Analysis of UMCRI

To confirm that UMCRI was an *S. aureus* species, UMCRI was analyzed in 95 separate biochemical reactions, used to phenotypically characterize *S. aureus* isolates (GP-2 microplate; Biolog, Inc., Hayward, CA). Minimum inhibitory concentrations (MICs) were determined for 27 different antibiotics by using the Clinical and Laboratory Standards Institute broth microdilution method.

Ribotyping was performed using the restriction enzyme *EcoRI* and an automated riboprinter (Qualicon-Dupont, Wilmington, DE). In addition, ordered restriction fragment mapping of the entire genome restricted with *XbaI*, was generated by OpGen, Inc. (Gaithersburg, MD). The ordered restriction map (optical map) of the UMCRI genome was compared with optical maps from 20 *S. aureus* clinical isolates and 14 *S. aureus* optical maps prepared in silico from whole-genome sequence data.

A first pass at whole-genome sequencing was performed by SeqWrite (Houston, TX). Full coverage of the genome was not achieved, although enough sequence data were generated to obtain the *arcC*, *gmk*, *pta*, and *yqiL* loci for analysis in multilocus sequence typing (MLST). Sequences for the *aroE*, *glpF*, and *tpi* loci were obtained by PCR and subsequent sequencing using primers (Invitrogen, Carlsbad, CA) *aroE*-Up, *aroE*-Dn, *glpF*-Up, *glpF*-Dn, *tpi*-Up, and *tpi*-Dn.²⁶ Typing and comparison of UMCRI was performed using the MLST *S. aureus* database and analysis software (www.mlst.net/ Multi Locus Sequence Typing/ provided in the public domain by Imperial College, London, UK).

UMCRI Comparison with MW2

Because of the genomic similarity between *S. aureus* strains UMCRI and MW2, experiments were performed to compare the virulence and pathogenicity of the two strains. The anterior chamber of rabbit eyes was infected with MW2 in the same fashion as described with group 1 and UMCRI. Growth of MW2 was quantified as CFU/mL of aqueous humor and disease was scored by SLE at 16 hours PI ($n = 8$). MICs for MW2 were also determined as described for UMCRI.

Statistical Analysis

The mean and SEM of slit lamp examination scores, colony-forming units/milliliter of aqueous, and PMNs were determined. PMN determinations, SLE scores, and the colony-forming units were compared by using analysis of variance and Student's *t*-tests between least-squared

means from each group (Excel; Microsoft, Redmond, WA). Differences at $P \leq 0.05$ were significant.

RESULTS

Bacterial Growth

Initially, each of seven different *S. aureus* strains (8325-4, Newman, 91-717, 3161-06, 30103, 177, and T1) in TSB was injected into the rabbit anterior chamber (group 1) and failed to grow. Extraction of the aqueous humor to remove a portion of the host defenses before injection (group 2) of these seven *S. aureus* strains also resulted in no bacterial growth. Protection of the bacteria from host defenses, by mixing the bacteria with a viscoelastic material (sodium hyaluronate) used in cataract surgery, was also tested (group 3). The viscoelastic material did not protect the bacteria from host defenses as evidenced by the failure of seven strains injected into the anterior chamber with the viscoelastic material to grow.

The *S. aureus* strain UMCR1 was injected, in the same fashion as other strains in group 1, into a normal rabbit eye. Unlike the other strains, UMCR1 was able to grow within the anterior chamber. To establish a minimal infectious dose of UMCR1 in the anterior chamber, rabbit eyes were injected with 10^3 or 10^4 CFU of UMCR1 in TSB. An inoculum of 10^4 CFU was found to be the lowest number of bacteria required to yield infection of all injected eyes. Bacteria were quantified at 2, 4, 6, 8, 12, and 16 hours after injection of 10^4 CFU. UMCR1 demonstrated steady bacterial growth throughout the course of infection that reached a maximum of $\log 6.97 \pm 0.18$ CFU/mL in the aqueous at 16 hours PI (Fig. 1A). Animals were not allowed to survive past 16 hours PI because of the severity of the infection.

Development of Disease

The progression of disease in rabbit eyes after injection of 10^4 CFU of UMCR1 was noted by the steady increase in SLE scores (Fig. 1B) over the course of the infection and was recorded by photographs (Fig. 2) taken at 2, 4, 6, 8, 12, and 16 hours PI. SLE scores reached a maximum of 14.03 ± 0.31 at 16 hours PI, a

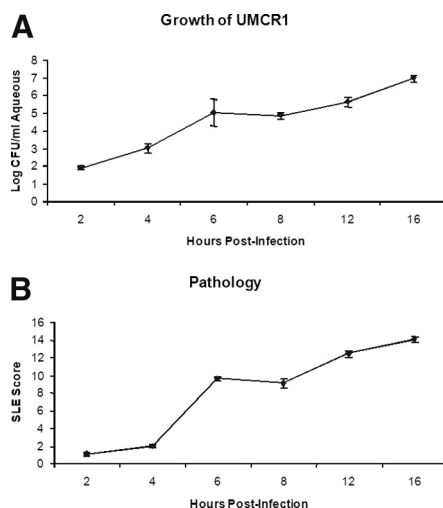


FIGURE 1. (A) The bacteria in aqueous humor samples were quantified at various time points after injection of 10^4 CFU *S. aureus* UMCR1 in $10 \mu\text{L}$ of TSB into the anterior chamber of rabbit eyes ($n \geq 4$ per time point). Continuous bacterial growth was observed throughout infection. (B) Rabbit eyes injected with 10^4 CFU of UMCR1 into the anterior chamber underwent SLE and were scored ($n = 4$ per time point) by two masked observers on the basis of disease present at various time points throughout infection.

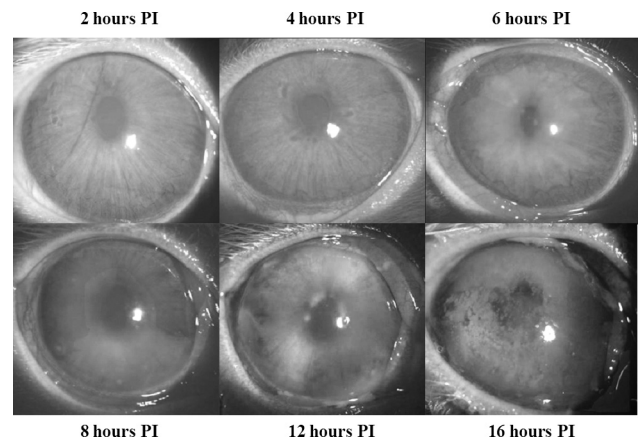


FIGURE 2. Rabbit eyes injected with UMCR1 into the anterior chamber were photographed at 2, 4, 6, 8, 12, and 16 hours PI to document changes in disease over time. Increased iritis, fibrin accumulation, and injection were noted as well as hemorrhaging of the iris at later time points.

score that in previous studies would not justify euthanization; however, because of hemorrhaging of the iris and an obvious increase in intraocular swelling, the animals were not allowed to continue with the infection.

The disease created by UMCR1 in this model of endophthalmitis was further characterized by determining the influx of PMNs into the anterior chamber by measuring MPO activity in the aqueous humor at 16 hours PI and comparing it with the MPO activity in aqueous humor from uninfected rabbit eyes. The MPO activity in the aqueous humor from infected eyes was equivalent to $\log 6.09 \pm 0.37$ PMNs, a value that was approximately 193-fold greater than the number of PMNs in uninfected aqueous humor ($\log 3.80 \pm 0.16$; $P = 0.0002$).

Histopathologic analysis of mock-infected and infected whole eyes removed at 16 hours PI was performed to determine the structures of the eye affected by the infection and the extent of endophthalmitis within the eye. In contrast to a mock-infected eye (Fig. 3, left), the tissue sections from eyes infected with UMCR1 (Fig. 3, right) at 16 hours PI had PMNs in the anterior chamber and the posterior chamber, as well as infiltration of the tissues of the iris, trabecular meshwork, and ciliary body. The tissues of the iris, ciliary body, and trabecular meshwork were also highly distorted due to edema. There was only moderate infiltrate into distal portions of the cornea and mild to moderate damage of the endothelium. There were no obvious signs of inflammation in the vitreous, retina, or choroid at 16 hours PI.

Properties of UMCR1

Biochemical testing of UMCR1 revealed metabolic reactions matching known *S. aureus* strains in 95 of 95 reactions tested (Table 1). MICs of 27 antibiotics for UMCR1 were determined, and all MIC values were typical of many *S. aureus* strains, including resistance to polymyxin B. However, UMCR1 was resistant to sulfamethoxazole, which is not typical of *S. aureus* (Table 2).

Comparison of the *EcoRI* ribopattern of UMCR1 with ribopatterns of other isolates produced a match with 39 known *S. aureus* strains (data not shown). Optical mapping produced an ordered *XbaI* restriction map of the UMCR1 genome, which was compared with that of 34 other *S. aureus* strains (Fig. 4). The UMCR1 genome was estimated to be 2.78 Mb in size and was most similar to strains MW2 and MSSA476, community-acquired isolates capable of causing severe human infections and whose genomes have been sequenced.^{27,28} By optical

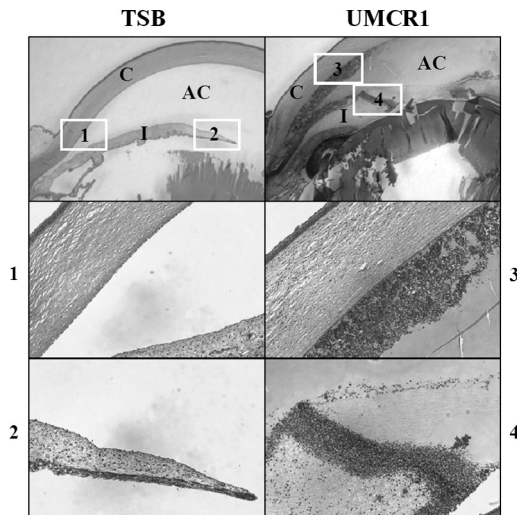


FIGURE 3. Whole rabbit eyes injected with UMCR1 (right) or TSB (left) into the anterior chamber were removed at 16 hours after injection and placed in a formaldehyde fixative solution and sectioned for histopathologic analysis. Higher magnification photographs correspond with the numbered boxes in the lower magnification images (top). (1) A mock-infected eye; (2) the iris of a mock-infected eye; (3) the PMN and fibrin accumulation within the anterior chamber of a UMCR1-infected eye; and (4) the inflammation and infiltration of PMNs into the tissues of the iris of a UMCR1-infected eye. C, cornea; AC, anterior chamber; I, iris.

mapping, UMCR1 had a 95% similarity to MW2 and MSSA476, whereas the similarity to the other 32 strains varied from 93% to 65%. Furthermore, optical mapping revealed that UMCR1 contains two inserts of 41 and 45 kb in size that are not present in the closely related MW2 or MSSA476 strains.

MLST of UMCR1 revealed a 1, 1, 40, 1, 1, 1, 1 allelic profile. All gene fragments matched *S. aureus* type ST 1 except the glycerol kinase fragment (*glpF*) which matched allele 40; the difference between alleles 1 and 40 of *glpF* resides in the substitution of a single nucleotide (base 129 of *glpF*). Strains MW2 and MSSA476 are both type ST 1, having an allelic profile of 1, 1, 1, 1, 1, 1, 1, when analyzed through MLST, and perfectly match UMCR1 in 3197 of 3198 nucleotides.

MW2 Endophthalmitis

Because the genomes of UMCR1 and MW2 were found to have extensive similarities, *S. aureus* strain MW2 was also tested for growth in the anterior chamber in the same fashion as strains in group 1 and UMCR1. The colony-forming units of pathogen per milliliter of aqueous and the disease observed at 16 hours PI after an MW2 injection into the anterior chamber was compared with that of UMCR1. MW2 in the aqueous humor 16 hours after injection was found to be $\log 0.71 \pm 0.27$ CFU/mL, a value significantly less than that of UMCR1 ($\log 6.97 \pm 0.18$, $P < 0.0001$). The SLE scores of MW2-injected eyes at 16 hours PI were 6.28 ± 0.78 , significantly lower than that of UMCR1-injected eyes at 16 hours PI (14.03 ± 0.31 ; $P < 0.0001$).

DISCUSSION

We characterized the first strain of *S. aureus* that is able to grow in the anterior chamber and described the infection in the rabbit. The model creates an infection analogous to that in human postcataract surgery, which is characterized by extensive bacterial growth and the development of severe disease. In the past, the powerful host defenses of the anterior chamber have prevented quantitative animal studies of *S. aureus* infec-

tions that mimic infections related to cataract or other anterior chamber surgeries in humans. Because of the potent host defenses, studying the effectiveness of therapies in the anterior chamber has been limited to the measurement of drug concentrations within the aqueous humor or antibiotic interactions with dying bacteria. The lack of a reliable animal model of an intact anterior chamber infection with *S. aureus* has also been a hindrance in the study of bacteria-host interactions, virulence mechanisms, and host defenses in endophthalmitis infections. Further studies using this new model with strain UMCR1 could provide much needed information on *S. aureus* anterior chamber infections.

The properties of the UMCR1 strain appear very different from those of any other *S. aureus* strains that have been studied in the anterior chamber. Other *S. aureus* strains do not appear to have the capability of surviving the innate host defenses present in the intact anterior chamber of the rabbit eye. Girgis et al.¹⁷ found that *S. aureus* underwent significant reductions in the number of viable bacteria after in vivo or in vitro exposure to rabbit aqueous humor. Furthermore, Maylath and Leopold²⁹ showed that *S. aureus* could not be isolated

TABLE 1. Identification of *S. aureus* by Biochemical Reactions

<i>S. aureus</i>	ATCC 25923*†	ATCC 49775*†	8325-4*†	UMCR1*†
Tween 40	—	—	+	—
Tween 80	—	—	+	—
<i>N</i> -acetyl-D-mannosamine	b	+	+	+
Arbutin	—	—	b	—
Cellobiose	—	+	b	—
D-Galactose	—	b	b	b
D-Gluconic acid	—	—	—	b
α -D-Lactose	—	—	b	—
D-Mannitol	+	+	b	+
D-Melezitose	+	+	+	b
α -Methyl D-glucoside	—	b	—	b
Palatinose	b	+	+	+
D-Raffinose	—	b	b	b
Sorbitol	b	+	b	+
Acetic acid	b	+	+	b
L-malic acid	—	—	+	—
Mono-methyl succinate	b	—	+	b
Succinamic acid	—	—	b	—
Succinic acid	+	—	+	b
D-Alanine	b	+	+	+
L-Alanine	b	+	+	+
L-Asparagine	—	—	b	+
L-Proglutamic acid	—	—	+	b
Glucose-1-phosphate	b	+	+	+
2,3-Butanediol	—	—	b	b

+, positive reaction; —, negative reaction; b, borderline.

* All strains tested were negative to water, glycogen, α -cyclodextrin, β -cyclodextrin, inulin, mannan, amygdalin, D-arabitol, L-fucose, D-galacturonic acid, gentiobiose, M-inositol, lactulose, D-melibiose, α -methyl D-galactoside, β -methyl D-galactoside, α -methyl D-mannoside, L-rhamnose, salicin, sedoheptulosan, stachyose, D-tagatose, xytilol, β -hydroxybutyric acid, γ -hydroxybutyric acid, *p*-hydroxyphenyl acetic acid, lactamide, D-lactic acid methyl ester, D-malic acid, propionic acid, *N*-acetyl L-glutaminic acid, alaninamide, and putrescine.

† All strains tested were positive to dextrin, *N*-acetyl-D-glucosamine, L-arabinose, D-fructose, α -D-glucose, maltose, maltotriose, D-mannose, D-xylose, 3-methyl glucose, β -methyl D-glucoside, D-psicose, D-ribose, sucrose, D-trehalose, turanose, α -hydroxybutyric acid, α -ketoglutaric acid, α -ketovaleric acid, glycyl-L-glutamic acid, L-lactic acid, methyl pyruvate, pyruvic acid, L-alanyl-glycine, L-glutamic acid, glycerol, adenosine, L-serine, 2'-deoxyadenosine, inosine, thymidine, uridine, adenosine-5'-monophosphate, thymidine-5'-monophosphate, uridine-5'-monophosphate, fructose-6-phosphate, glucose-6-phosphate, and D-L- α -glycerol phosphate.

TABLE 2. Minimum Inhibitory Concentrations of Antibiotics

	UMCR1	MW2
Amikacin	4	8
Amoxicillin	0.25	64
Ampicillin	0.125	64
Azithromycin	1	1
Cefazolin	0.5	4
Ceftazidime	8	16
Chloramphenicol	8	16
Ciprofloxacin	0.5	0.5
Clindamycin	0.125	0.125
Erythromycin	0.25	0.5
Fusidate	0.125	0.125
Gentamicin	0.5	0.25
Imipenem	≤0.125	≤0.125
Minocycline	0.125	0.25
Moxifloxacin	0.06	0.06
Neomycin	2	2
Ofloxacin	0.5	0.5
Oxacillin	0.25	16*
Penicillin	0.031	64
Piperacillin	1	128
Polymyxin B	64†	64
Rifampin	≤0.015	≤0.015
Sulfamethoxazole	>512‡	256
Tetracycline	0.5	1
Tobramycin	0.5	0.25
Trimethoprim	4	4
Vancomycin	1	1

Data are micrograms/milliliter.

* Confirms methicillin resistance.

† Typical resistant value for *S. aureus*.

‡ Unusual resistance for *S. aureus*.

from aqueous humor after injection into the anterior chamber of rabbits. However, other studies demonstrate some strains of *S. aureus* that can at least survive in minimal numbers in the anterior chamber. Koul et al.³⁰ found that rabbit eyes injected with *S. aureus* yielded 21 positive cultures in 64 aqueous humor samples. Similarly, Kowalski et al.^{18,19} and de Castro et al.²⁰ described models of *S. aureus* endophthalmitis in which viable bacteria were recovered from the aqueous humor of some injected eyes, but there was no consistent survival of bacteria or consistent bacterial growth in eyes with surviving bacteria. The study presented herein demonstrates the advantage of strain UMCR1 not only to survive consistently, but to grow reliably after injection into the intact anterior chamber.

Another important and relevant characteristic of this organism is its ability to cause severe pathogenesis during infection of the anterior chamber. Studies have demonstrated the ability of certain *S. aureus* strains to cause significant inflammation by 24 hours after injection of 10^3 to 10^6 CFU into the anterior chamber, even though bacteria did not grow or even survive.^{17–20} However, the amount of inflammation and disease produced by these organisms in the anterior chamber was highly variable, even when the same strain of bacteria was used.^{18–20} In contrast, the UMCR1 model consistently produced extensive disease and tissue damage as observed in the SLE scores, photographs, and histology of the infected eyes. In further contrast, infection with UMCR1 caused such severe disease, such as hemorrhaging of the iris, that the infection had to be terminated at only 16 hours PI.

Although the disease caused by UMCR1 in the anterior chamber was severe, the disease observed in the retina and vitreous was minimal at 16 hours PI, perhaps because the bacteria and pathogenic factors they produce are impeded from reaching the posterior segment by the natural barriers in a normal eye such as the lens, iris, and flow of aqueous humor.

The pathologic outcome of an anterior chamber infection is different from that observed in endophthalmitis models that are initiated by injecting *S. aureus* into the vitreous. Intravitreal infections require 24 to 48 hours to demonstrate the significant inflammation and pathologic changes that occur in both the posterior and anterior segments of the eye.³¹ Apparently, the prolonged infection in the vitreous allows the spread of pathogenic factors and the inflammatory processes to the anterior portions of the eye.

Another important difference between anterior chamber and intravitreal infections is that many *S. aureus* strains are capable of surviving and growing within the vitreous humor, whereas they typically die in the anterior chamber. The ease of establishing an intravitreal infection indicates that the vitreous lacks an important host defense that is present in the aqueous humor and/or anterior chamber. The infection of the anterior chamber with UMCR1 demonstrates the survival and growth of *S. aureus* while being exposed to these powerful host defenses.

Because *S. aureus* UMCR1 is unique in its ability to resist the ocular host defenses and to grow and cause severe disease in the anterior chamber, MLST and genetic mapping studies were performed to provide some insight into the relation of UMCR1 to other *S. aureus* strains. The data from MLST and optical mapping of restriction enzyme fragments from UMCR1 and other strains revealed its close relation to MSSA476 and MRSA strain MW2. Both MSSA476 and MW2 have been described as agents of severe infection that can be lethal in humans.^{27,28,32,33} Optical genetic mapping also revealed two regions in the UMCR1 genome that are not found in MW2 or MSSA476. The unknown regions within UMCR1 could be a key to its resistance of host defenses and ability to cause highly damaging infections in the anterior chamber. Furthermore, UMCR1 differs from MW2 in that it lacks an SCCmec cassette.

The fact that strain UMCR1 was isolated from a rabbit does not mean that it is rabbit specific. *S. aureus* strains are thought to be shared between humans and rabbits.^{34,35} Also, the data from the genetic analysis of UMCR1 demonstrating its extensive similarity to two human isolates (MW2 and MSSA476) and other ST1 typed *S. aureus* lends support to this idea. Although MW2 and MSSA476 have not been noted in ocular infections, their extensive genetic similarities with UMCR1 warranted further study. Therefore, the ability of MW2 to infect the rabbit

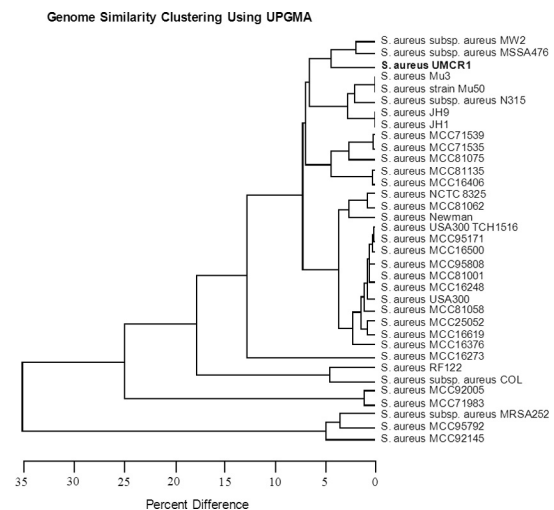


FIGURE 4. Similarity of UMCR1 compared with clinical and in situ *S. aureus* strains, according to the optical map of ordered *Xba*I restrictions. (The genome of UMCR1 is most similar to that of MW2, an *S. aureus* isolate with a fully sequenced genome).

anterior chamber was tested. After inoculation of the rabbit anterior chamber, MW2 was unable to grow and did not cause severe ocular disease, unlike UMCRI. These results demonstrate the unusual virulence and pathogenic capabilities of UMCRI, the mechanisms of which have yet to be demonstrated.

Pore-forming toxins, such as α -toxin, are known to be major contributors to staphylococcal virulence and pathogenesis in ocular infections.^{8,21,22} The unprecedented growth and pathogenesis of UMCRI within the anterior chamber could indicate an increased amount of toxin expression of UMCRI compared with other *S. aureus* strains. However, preliminary studies using hemolysis assays to measure the toxin production of *S. aureus* culture supernatants indicate that UMCRI toxin expression was comparable to or less than that of strains such as MW2 and 8325-4, which do not survive within the rabbit anterior chamber.¹⁷ These data suggest that toxin production is not a key mechanism for the survival of *S. aureus* within the anterior chamber.

The present data demonstrate that one *S. aureus* strain withstood the host defenses and replicated in rabbit aqueous humor, whereas all other isolates tested in this study or elsewhere did not. There are no data to explain this difference. Among the possibilities to consider is a genetic alteration in UMCRI that allows production or overproduction of a virulence-related component or the acquisition of an unusual virulence determinant. The virulence characteristics that only UMCRI appears to express may affect the establishment of an *S. aureus* infection such as those seen in postcataract surgery-related endophthalmitis.

This study presents a reproducible rabbit model of endophthalmitis caused by an *S. aureus* infection in the intact anterior chamber. The model described was shown to more closely mimic the bacterial growth and disease in clinical infections of postcataract endophthalmitis than did previous models of anterior chamber infection. The ability to quantify bacterial growth in this model establishes the presence of an active infection that is expected to be useful for studying bacterial pathogenesis, host defenses, and the effectiveness of therapies within the anterior chamber.

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