Staphylococcus aureus Isolated from Endophthalmitis Are Hospital-Acquired Based on Panton-Valentine Leukocidin and Antibiotic Susceptibility Testing

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Abstract

Purpose: *Staphylococcus aureus* (SA) endophthalmitis is generally a postsurgical infection with an undefined source of entry. Hospital-acquired (HA) SA infections are associated with multi-antibiotic resistance and absence of the Panton-Valentine Leukocidin (PVL) toxin. Community-acquired (CA) SA infections are not associated with multi-antibiotic resistance and possess the PVL toxin. We hypothesize that CA infection is more common than HA for SA endophthalmitis.

Methods: Twenty de-identified SA isolates, collected from the vitreous and/or aqueous of clinical endophthalmitis, were tested for the presence of PVL toxin and antibiotic susceptibility. PVL testing was performed using a kit to detect the Staphylococcal toxin by reversed passive latex agglutination (PVL-RPLA “Seiken,” Denka Seiken Co., LTD). SA isolates were tested for antibiotic susceptibility using disk diffusion at the time of isolation. Multi-antibiotic resistance was defined as resistance to at least 3 classes of antibiotics.

Results: Of the 20 isolates, 15 were multi-antibiotic resistant and PVL-negative consistent with HA, and 1 was not multi-antibiotic resistant and PVL-positive, consistent with CA. Two isolates tested positive for PVL with one demonstrating both methicillin and fluoroquinolone (FQ) resistance. Of the 18 PVL-negative SA isolates, 15 (83%) were multi-antibiotic resistant (12 methicillin-resistant SA, 14 FQ resistant).

Conclusions: Our results reject the hypothesis that SA isolated from endophthalmitis is consistent with CA sources due to the lack of the PVL toxin and multiple resistant patterns of the SA. PVL does not appear to be a key virulence factor for the development of SA endophthalmitis.

Introduction

ENDOPHTHALMITIS IS A SERIOUS infection of the eye, and if not appropriately treated, may lead to permanent vision loss and even loss of the infected eye. A patient may develop bacterial endophthalmitis as a result of systemic infection (endogenous bacterial endophthalmitis), after a penetrating trauma to the eye, or after ocular surgery such as cataract extraction.¹ Although rare, occurring in only 0.07%–0.32% of cases, endophthalmitis after cataract extraction is most frequently a result of coagulase-negative staphylococcal infection.² At the University of Pittsburgh Medical Center, Pittsburgh, PA, coagulase-negative *Staphylococcus* and *Staphylococcus aureus* (SA) account for ~57.7% and 10.5% of endophthalmitis infections, respectively (Fig. 1). This would give the indication, that after cataract surgery with an endophthalmitis rate of 0.1%, the incidence of SA endophthalmitis would be 1 in 10,000 of surgical cases.

SA is known to produce a variety of virulence factors, and this ability is largely responsible for its resilience, as well as the diversity of disease, that SA can cause. Virulence factors such as leukocidins, hemolysins, and hyaluronidase promote the spread of SA in host tissues and damage host cell membranes, thereby leading to host cell lysis. Protein A, carotenoids, and catalase inhibit phagocytosis and/or enhance survival of SA within phagocytes. Enterotoxins and exfoliative toxins, implicated in acute-onset food poisoning as well as toxic shock syndrome and staphylococcal scalded skin syndrome, respectively, also contribute to host cell destruction.³

Epidemiologically, SA can be divided into 2 groups based on genetic characteristics, antibiotic resistance, and the presence of virulence factors such as Panton-Valentine Leukocidin (PVL). PVL is a leucotoxin with the ability to lyse leukocytes that is associated with soft-tissue and skin infection.⁴ Several studies over the past decade have shown that

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FIG. 1. Distribution of bacteria isolated from endophthalmitis (1993–2010) ($n=518$) provided by the Charles T. Campbell Ophthalmic Microbiology Laboratory, Pittsburgh, PA.

the PVL toxin genes are frequently expressed in community-acquired infection SA (CA-SA) strains and almost universally absent from hospital-acquired infection SA strains (HA-SA).^{4–6} HA-SA tend to be resistant to multiple antibiotic classes, whereas CA-SA are more broadly susceptible to antibiotics.^{4,7–10} To date, there has been no specific study that evaluates the origin of SA implicated in endophthalmitis for the PVL gene products.

We hypothesize that SA strains causing bacterial endophthalmitis are CA strains and predict that all our SA endophthalmitis isolates will test positive for PVL and not display multi-resistance to antibacterial drugs.

Methods

Bacterial isolates

Twenty SA isolates were tested for the presence of PVL toxin and antibiotic susceptibility. The isolates were de-identified frozen stocks (-80°C) consecutively collected from the vitreous and/or aqueous of patients with clinical endophthalmitis from June 2000 to December 2010 at the Charles T. Campbell Ophthalmic Microbiology Laboratory, University of Pittsburgh Medical Center, Pittsburgh, PA. These isolates were a part of the clinical bank collection used to validate new antibiotics and diagnostic testing, and monitoring of antibiotic resistance. Only vitreous and/or aqueous cultures that were robustly positive on 2 or more culture media were included in the study. SA from cultures that tested positive only in a single broth or culture plate was excluded to assure that a remote contaminant was not included in the study. As de-identified isolates, no patient personal or medical record clinical data were reviewed for the SA isolates; thus, the nature and origin of the SA endophthalmitis are not known.

In vitro antibiotic susceptibility testing

The 20 SA isolates were tested for antibiotic susceptibility using disk diffusion at the time of isolation. There are no standards for interpreting topical treatment, but the serum standards can be used if it is assumed that the antibiotic concentrations in the ocular tissue are equal or greater than the antibiotic concentrations which can be attained in the serum.^{11,12} The earlier National Committee for Clinical Labora-

tory Standards (now CSLI, Clinical and Laboratory Standards Institute, Wayne, PA) methods were used to interpret susceptibility using standards based on serum concentrations.¹¹ The CSLI standards have changed to accommodate the refinement of serum-level susceptibility, but these do not reflect any change for topical therapy and have no bearing on the ophthalmic interpretation. In fact, an increase in resistance will prevail with the updated standards using the same susceptibility zones of the test isolates. Experience (33 years) has dictated that intermediate susceptibility has no meaning for ocular isolates, and high antibiotic concentrations in the ocular tissue should interpret intermediate as susceptible. In addition, the previous existing vancomycin disk diffusion standard is still reliable for testing the susceptibility of ocular bacterial isolates. The use of previous disk diffusion zones for vancomycin allows for more practical testing and has not compromised patient care. Moxifloxacin and gatifloxacin were not available for treatment options for the first 9 SA isolates. All 9 were retrospectively tested at the time of this study with the disk diffusion method. All bacteria isolated from endophthalmitis are routinely tested for susceptibility to vancomycin, gentamicin, ciprofloxacin, ofloxacin, cefazolin, amikacin, ceftazidime, ampicillin, clindamycin, gatifloxacin, moxifloxacin, and methicillin. Initially, methicillin resistance was determined by interpreting the susceptibility to oxacillin, but this is now determined with cefoxitin.

PVL detection

PVL testing was performed using a kit to detect the Staphylococcal toxin by reversed passive latex agglutination (PVL-RPLA “Seiken, Denka Seiken Co., LTD, Tokyo, Japan”).¹³ The kits were not available for purchase and were kindly provided by Denka Seiken Co., LTD. This test utilizes latex particles that have been sensitized with antibodies to PVL and can distinguish between PVL-positive and PVL-negative bacterial strains on the basis of agglutination with sensitized latex particles. This test has been shown to be both 100% sensitive and specific when compared with polymerase chain reaction (PCR) studies to evaluate for the presence of PVL genes.¹³ Additionally, this kit confers the immediate advantage, over conventional PCR techniques, of providing quantitative information about the amount of PVL gene products that are produced by the bacteria.^{14,15}

TABLE 1. BACTERIAL SUSCEPTIBILITY OF 20 *STAPHYLOCOCCUS AUREUS* ENDOPHTHALMITIS ISOLATES AS DETERMINED BY DISK DIFFUSION USING NATIONAL COMMITTEE FOR CLINICAL LABORATORY STANDARDS

ID#	PVL	Vancomycin	Gentamicin	Ciprofloxacin	Ofloxacin	Cefazolin	Anikacin	Ceftazidime	Ampicillin	Clindamycin	Oxacillin	Gatifloxacin	Moxifloxacin
E311	Neg	S 17	S 23	R 0	R 8	S 22	S 17	R 12	R 12	R 0	R 0	R 12	R 15
E314	Neg	S 18	S 25	R 0	R 0	S 30	S 16	R 15	S 31	R 0	R 10	R 11	R 12
E325	Neg	S 14	R 0	R 0	R 0	R 0	R 12	R 0	R 0	R 0	R 0	R 13	R 14
E327	Neg	S 20	S 22	R 0	R 9	S 19	S 20	R 12	R 10	R 0	R 0	R 14	S 16
E331	Neg	S 18	S 20	S 21	S 25	S 26	S 19	S 23	R 17	S 25	S 19	S 29	S 31
E332	Neg	S 17	S 21	R 0	R 7	S 40	S 21	S 33	S 39	S 22	S 26	R 14	R 15
E333	Neg	S 16	S 19	R 0	R 0	R 14	R 13	R 12	R 10	R 0	R 0	R 14	R 15
E369	Neg	S 16	S 18	S 22	S 20	S 24	S 22	S 18	R 13	S 24	S 14	S 27	S 27
E379	Neg	S 17	S 21	R 0	R 0	S 20	R 13	R 11	R 9	R 0	R 0	R 14	R 15
E386	Neg	S 18	S 20	R 0	R 0	S 21	S 20	R 14	R 14	S 25	S 13	R 12	S 16
E401	Neg	S 18	S 20	R 0	R 0	S 21	R 14	S 17	R 11	R 0	S 15	R 9	R 11
E414	Neg	S 19	S 24	R 0	R 0	S 19	S 15	R 0	R 10	R 0	R 0	S 21	S 25
E424	Neg	S 18	S 21	R 0	R 0	S 21	S 15	S 16	R 10	R 0	S 16	R 10	S 14
E425	Neg	S 18	S 22	R 0	R 0	R 0	S 21	R 0	R 10	S 27	R 0	R 9	R 12
E427	Neg	S 16	S 22	R 0	R 0	R 10	S 18	R 0	R 8	S 26	R 0	R 7	R 10
E442	Neg	S 15	S 23	R 0	R 0	R 10	S 15	R 14	R 0	R 0	R 11	R 11	R 11
E475	Neg	S 17	S 17	R 0	R 0	S 20	R 11	R 14	R 6	R 0	R 11	S 16	S 16
E504	Neg	S 18	S 19	R 0	R 0	R 10	R 13	R 0	R 6	R 0	R 0	R 7	R 7
E532	POS	S 18	S 17	R 0	R 6	S 20	R 13	R 6	R 16	R 22	R 0	R 14	S 16
E572	POS	S 18	S 22	S 26	S 24	S 29	S 22	S 18	R 18	S 26	S 25	S 24	S 25
% Susceptible	100% (20/20)	95% (19/20)	15% (3/20)	15% (3/20)	15% (3/20)	70% (14/20)	65% (13/20)	30% (6/20)	10% (2/20)	35% (7/20)	35% (7/20)	25% (5/20)	40% 8/20

Numbers indicate zone of inhibition.

R, resistant; S, susceptible; PVL, Pantone-Valentine leukocidin.

The SA isolates were retrieved from the frozen stocks (−80°C) by plating with a loop on trypticase soy agar supplemented with 5% sheep blood (BBL™; Becton, Dickinson and Co., Sparks, MD) and incubating in a CO₂ atmosphere overnight at 36°C. Each SA isolate was inoculated to 5 mL of Brain Heart Infusion medium (Bacto™; Becton, Dickinson and Co.) and allowed to be shaken at 37°C for ~20 h. After this incubation period, each sample was centrifuged at 3,000 rpm for 20 min, and the supernatant was used for PVL testing.

PVL testing was performed according to the manufacturer's instructions (kit insert), which clearly designated that testing was "For Research Use Only. Not for use in diagnostic procedures." Pipettes and pipette tips to deliver 25 µL of reagent were necessary. A 12-channel multi-pipettor was advantageous for performing dilutions. All testings were performed in 96-well cell culture cluster v-bottom polystyrene sterile plates (Costar®; Corning Inc., Corning, NY).

In addition to reading plates against a black background, all plates were read against a light box and compared with the agglutination patterns provided with the PVL detection kit. The PVL results were tabulated for positive and negative agglutination patterns.

All testing was repeated to confirm consistent reproducible results.

Results

Table 1 summarizes the bacterial susceptibility of 20 endophthalmitis isolates to commonly used antibacterial drugs. Of the 13 methicillin-resistant SA (MRSA), 11 (85%) were fluoroquinolone (FQ)-resistant, and 2 (15%) were FQ-susceptible. Of the 7 methicillin-sensitive SA (MSSA), 4 (57%) were FQ-resistant, and 3 (43%) were FQ-susceptible. Fifteen (75%) of the 20 SA isolates were resistant to both the second-generation FQs (ciprofloxacin and ofloxacin) and the fourth-generation FQs (moxifloxacin and gatifloxacin). Two (10%) of the 20 SA isolates were susceptible to the fourth-generation FQs but resistant to the second-generation FQ. Three (15%) of the 20 SA isolates were susceptible to both the second- and fourth-generation FQs.

Table 2 summarizes the descriptive statistics of 20 PVL Tested SA isolated from endophthalmitis. Of the 20 isolates, 15 were multi-antibiotic resistant and PVL-negative consistent with HA, and 1 was not multi-antibiotic resistant and PVL-positive, consistent with CA. Only 2 isolates tested positive for PVL with one demonstrating methicillin and FQ resistance. The agglutination pattern was positive for the first 2 dilutions of one isolate, and only for one dilution for the second isolate, thus suggesting a low load of PVL toxin for both isolates. Of the 18 PVL-negative SA isolates, 15 (83%) were multi-antibiotic resistant (12 MRSA, 14 FQ resistant), and 3 (17%) did not display resistance to methicillin and the FQ anti-infectives.

Discussion

Coagulase-negative *Staphylococcus* is both a common cause of endophthalmitis after cataract surgery and a part of the normal colonizing bacterial flora of the eyelid.^{16,17} Speaker-reported supporting data that are genetically identical to coagulase-negative *Staphylococcus* from the eyelid have also been isolated from the vitreous of the same

TABLE 2. DESCRIPTIVE STATISTICS OF 20 PANTON-VALENTINE LEUKOCIDIN TESTED *STAPHYLOCOCCUS AUREUS* ISOLATED FROM ENDOPHTHALMITIS

Susceptibility profile	PVL positive (n=2)	PVL negative (n=18)
Antibiotic Multi-resistant SA	1	16
MRSA	1	12
MSSA	1	6
SA (ciprofloxacin, ofloxacin resistant)	1	16
SA (gatifloxacin, moxifloxacin resistant)	1	14
MRSA fluoroquinolone resistant	1	12
MRSA fluoroquinolone susceptible	0	0
MSSA fluoroquinolone resistant	0	4
MSSA fluoroquinolone susceptible	1	2

SA, *Staphylococcus aureus*; MRSA, methicillin-resistant SA; MSSA, methicillin-sensitive SA.

patient.¹⁸ Given that SA may be found as a normal eyelid commensal, it is reasonable to propose an auto-acquired route from a patient's normal flora.¹⁶ Whether SA endophthalmitis is uniquely auto-acquired from the eyelid, however, is another question. Although SA skin infections or pneumonia, for example, can be classified as either HA or CA, this distinction is less clear in SA infections of the eye, including endophthalmitis. The majority of SA isolates in our study demonstrated multi-drug resistance that is not known to be characteristic of typical eyelid SA. It seems highly unlikely that healthy patients undergoing routine ocular surgery harbor multi-resistant SA as a part of their normal flora or that their antibiotic-susceptible SA acquired resistance in the short perioperative period. We propose, therefore, that the SA causative of endophthalmitis is most likely introduced as nosocomial pathogens at the time of surgery.

A recent paper by Hesje et al. described the molecular epidemiology of MRSA and MSSA isolated from the conjunctiva, cornea, aqueous humor, and vitreous.⁶ They characterized 38 isolates, and determined that 22 were HA strains which possessed the *SCCmecII* cassette for beta lactam resistance. All 22 HA isolates were PVL negative when tested with PCR. The 16 remaining isolates were *SCCmecIV* positive CA strains, 75% of which were positive for PVL. Although this study looked exclusively at methicillin-resistant bacteria and did not specifically investigate endophthalmitis, the results of our study are congruent in that the majority of ocular SA are multi-drug resistant, PVL negative bacteria. Although the results of our study suggest that PVL is not a necessary virulence factor for SA endophthalmitis, this cytotoxin has been shown to confer greater virulence to SA infections elsewhere in the body.^{6,8,9,18}

Clinically, an important finding of this study is the determination that the majority of our SA isolates are resistant to second- and fourth-generation FQ anti-infectives as well as to methicillin. This is of great clinical importance, as these drugs are frequently given for prophylaxis before and after routine cataract extraction. Although it has been demonstrated in a rabbit model that a fourth-generation FQ can still be effective against an *in vitro* resistant SA¹⁹ in the prevention of endophthalmitis, these anti-infectives may be less

prudent to use based on *in vitro* susceptibility testing.²⁰ In light of the paucity of novel antibacterial drugs, it now becomes necessary to consider infection prevention in the surgical setting rather than focusing on treating a postsurgical complication.

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Author Disclosure Statement

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