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Susceptibility of ocular *Staphylococcus aureus* to antibiotics and multipurpose disinfecting solutions.

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Abstract: *Staphylococcus aureus* is a frequent cause of ocular surface infections worldwide. Of these surface infections, those involving the cornea (microbial keratitis) are most sight threatening. *S. aureus* can also cause conjunctivitis and contact lens-related non-infectious corneal infiltrative events (niCIE). The aim of this study was to determine the rates of resistance of *S. aureus* isolates to antibiotics and disinfecting solutions from these different ocular surface conditions. 63 *S. aureus* strains from the USA and Australia were evaluated; 14 from niCIE, 26 from conjunctivitis and 23 from microbial keratitis (MK). The minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) of all the strains to ciprofloxacin, ceftazidime, oxacillin, gentamicin, vancomycin, chloramphenicol, azithromycin and polymyxin B was determined. The MIC and MBC of the niCIE strains to contact lens multipurpose disinfectant solution (MPDS) was determined. All isolates were susceptible to vancomycin (100%). The susceptibility to other antibiotics decreased in the following order: gentamicin (98%), chloramphenicol (76%), oxacillin (74%), ciprofloxacin (46%), ceftazidime (11%), azithromycin (8%) and polymyxin B (8%). 87% of all isolates were multidrug resistant and 17% of isolates from microbial keratitis were extensively drug resistant. The microbial keratitis strains from Australia were usually susceptible to ciprofloxacin (57% vs. 11%; $p = 0.04$) and oxacillin (93% vs. 11%; $p = 0.02$) compared with microbial keratitis isolates from the USA. Microbial keratitis isolates from the USA were less susceptible (55%) to chloramphenicol compared with conjunctivitis strains (95%; $p = 0.01$). Similarly, 75% of conjunctivitis strains from Australia were susceptible to chloramphenicol, compared with 14% of microbial keratitis strains ($p= 0.04$). Most (93%) strains isolated from contact lens wearers were killed in 100% MPDS, except *S. aureus* 27. OPTI-FREE PureMoist was the most active MPDS against all strains with 35% of strains having an MIC ≤ 11.36%. There was a significant difference in susceptibility between OPTI-FREE PureMoist and Biotrue ($p = 0.02$). *S. aureus* non-infectious CIE strains were more susceptible to antibiotics than conjunctivitis strains and conjunctivitis strains were more susceptible than microbial keratitis strains. Microbial keratitis strains from Australia were more susceptible to antibiotics in comparison with microbial keratitis strains from the USA. Most of the strains were multi-drug resistant. There was variability in the susceptibility of contact lens isolates to MPDS, one strain *S. aureus* 27 from niCIE in Australia, was highly resistant to all 4 MPDS and 3 different types of antibiotics. Knowledge of the rates of resistance to antibiotics in different conditions and regions could help guide treatment of these diseases.

Keywords: *Staphylococcus aureus*; microbial keratitis; conjunctivitis; corneal infiltrative events; antibiotic susceptibility, MPDS susceptibility.

Introduction

S. aureus is one of the most common cause of ocular infections worldwide [1]. It has been reported as the most common cause of microbial keratitis (MK), a sight threatening infection of the cornea [2] in Australia [3,4] and the USA, [5,6]. Conjunctival infection (conjunctivitis) is also frequently caused by *S. aureus* [7]. *S. aureus* is also commonly observed in inflammatory adverse reactions associated with contact lens wear. These corneal infiltrative events are differentiated into infections or inflammatory conditions; the latter collectively called non-infectious corneal infiltrative events (niCIE) [8].

Treatment of MK involves the intensive use of topical antibiotics, commonly monotherapy with fluoroquinolones or use of fortified antibiotics (for example a beta lactam such as cefazolin with an aminoglycoside such as tobramycin or gentamicin) [9,10]. Conjunctivitis may be treated by topical application of tetracycline, chloramphenicol or fluoroquinolones [11]. Conversely non-infectious corneal infiltrative events (niCIEs) are self-limiting and resolve upon removal of the contact lens, although prophylactic treatment with topical broad-spectrum antibiotics such as fluoroquinolones, chloramphenicol and polymyxin B with low dose topical steroids [12] may be used.

S. aureus infections can be difficult to treat because strains may be resistant to multiple antibiotics. *S. aureus* has the ability to acquire resistance to virtually every antibiotic that has entered clinical use [13]. Increasing antimicrobial resistance of *S. aureus* has been identified as a public health threat by the World Health Organization [14]. Since emerging in 1961, the incidence and prevalence of methicillin-resistant *S. aureus* (MRSA) in ocular infections has increased dramatically [15,16]. Antibiotic resistance in *S. aureus* can be both inherited and acquired. Inherited resistance [17] includes genes naturally present on chromosomes which confer low membrane permeability, efflux pump expression and enzymatic inactivation of antibiotics [18]. Acquired resistance includes genetic mutations [19] and horizontal transfer of genes across the strains via mobile genetic elements [20].

Contact lens multipurpose disinfectant solutions (MPDS) are used to disinfect contact lenses when they are not being worn. MPDS contain disinfectants such as quaternary ammonium compounds or biguanides. *S. aureus* strains which possess *qac* genes can be resistant to disinfectants and are more commonly resistant to antibiotics [14]. As *qac* genes occur along with genes for antibiotic resistance, there is concern that resistance to disinfectants may increase the spread of antibiotic resistance [21].

There is limited information available on antimicrobial and MPDS susceptibility patterns of clinical isolates of *S. aureus* from Australia in comparison to other countries. The purpose of this study was to investigate the antibiotic and MPDS sensitives of *S. aureus* isolates from different ocular surface conditions isolated in Australia and the USA.

Results

Antibiotic susceptibilities

Table 1. summarizes the MIC and MBC of *S. aureus* strains to antibiotics. All isolates were susceptible to vancomycin (100%). The susceptibility to the other antibiotics decreased in the following order: gentamicin (98%), chloramphenicol (76%), oxacillin (74%), ciprofloxacin (46%), ceftazidime (11%), azithromycin (8%) and polymyxin B (8%). Most of the microbial keratitis strains from Australia were more commonly susceptible to ciprofloxacin (57%) and oxacillin (93%) compared with microbial keratitis strains from the USA for ciprofloxacin (11%; p= 0.04) and oxacillin (11%; p=0.02).

Chloramphenicol susceptibility varied by ocular condition and origin of the isolates. 95% of conjunctivitis and 78% of non-infectious CIE strains from Australia were susceptible to chloramphenicol. There was a significantly lower rate of susceptibility of microbial keratitis strains from Australia (14%) compared with Australian conjunctivitis strains (95%; p= 0.04). There was a similar pattern amongst the USA isolates, with 55% of the microbial keratitis strains and 95% of the conjunctivitis strains being sensitive to chloramphenicol. Overall, 30 % of microbial keratitis strains from Australia and the USA were susceptible to chloramphenicol than conjunctivitis and non-infectious CIE strains (85%; p=0.01).

Most strains (87%; 55/63) were multi-drug resistant (MDR), defined as resistant to three different classes of antibiotics [22]. Strains 111, 112, 113 from the USA (microbial keratitis) and M43-01 from Australian (microbial keratitis) group were extensively drug resistant (XDR) strains, defined as resistant to almost all antibiotics classes [22]. Strain 32 from Australia (niCIE) and 46 from the USA (conjunctivitis) were susceptible to all antibiotics used. Strains from niCIE were more susceptible to antibiotics compared with strains from infections (conjunctivitis + microbial keratitis). The susceptibility of microbial keratitis strains varied by origin of isolates, with microbial keratitis *S. aureus* strains from the USA being more likely to be MRSA and multidrug resistant compared with Australian microbial keratitis strains.

Antibiotics (sensitive, intermediate, resistant; µg/ml)																	
Ocular condition	**Strains**	**Ciprofloxacin ≤1, 2, ≥ 4* µg/ml**		**Ceftazidime ≤8, 16, ≥ 32 µg/ml**		**Oxacillin ≤ 2, ≥ 4 µg/ml**		**Gentamicin ≤4, 8, ≥16 µg/ml**		**Vancomycin ≤2, 4-8, ≥16 µg/ml**		**Chloramphenicol ≤8, 16, ≥ 32 µg/ml**		**Azithromycin ≤2, 4 ≥ 8 µg/ml**		**Polymyxin B ≤2, 4, ≥ 8**	
		MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Microbial keratitis USA	**106**	128 (R)	256	128 (R)	256	8 (R)	16	1 (S)	2	0.5 (S)	1	8 (S)	16	16 (R)	32	40 (R)	16
	107	64 (R)	128	64 (R)	128	128 (R)	256	0.5 (S)	1	0.5 (S)	1	16 (S)	32	16 (R)	32	160 (R)	32
	108	1 (S)	2	64 (R)	128	32 (R)	32	1 (S)	2	0.5 (S)	1	8 (S)	16	8 (R)	16	4 (R)	8
	109	128(R)	256	128 (R)	256	128 (R)	256	1 (S)	2	0.5 (S)	1	8 (S)	16	16 (R)	32	4 (I)	8
	110	128(R)	256	64 (R)	128	128 (R)	256	0.5 (S)	1	0.5 (S)	1	8 (S)	16	128(R)	256	80 (R)	16
	111	1280 (R)	2560	32 (R)	64	128 (R)	256	8 (I)	16	2 (S)	2	32 (R)	64	128 (R)	256	80 (R)	16
	112	2560 (R)	5120	32 (R)	64	128 (R)	256	2 (S)	4	1 (S)	2	32 (R)	64	128 (R)	256	320 (R)	640
	113	1280 (R)	2560	32 (R)	64	32 (R)	64	1 (S)	2	1 (S)	1	32 (R)	64	320(R)	1280	4 (I)	8
	114	8 (R)	16	16 (I)	32	0.5 (S)	1	4 (S)	8	0.5 (S)	1	32 (R)	64	640 (R)	1280	8 (R)	16
Microbial keratitis AUS	**34**	1 (S)	2	64 (R)	64	1 (S)	1	1 (S)	2	1 (S)	1	8 (S)	16	16 (R)	32	128 (R)	256
	129	1 (S)	1	16 (I)	32	0.5 (S)	1	0.5 (S)	1	2 (S)	2	32 (R)	64	32 (R)	64	40 (I)	16
	M5-01	64 (R)	128	128 (R)	256	2 (S)	4	2 (S)	2	1 (S)	2	128 (R)	256	8 (R)	16	128 (R)	256
	M19-01	1 (S)	2	128(R)	256	0.5 (S)	1	2 (S)	2	1 (S)	2	2 (S)	4	16 (R)	32	128 (R)	256

	M27-01	1 (S)	2	128 (R)	256	0.5 (S)	1	2 (S)	4	1 (S)	2	128(R)	256	128(R)	256	128 (R)	256
	M28-01	1 (S)	2	320 (R)	640	0.5 (S)	1	0.5 (S)	1	1 (S)	2	16 (I)	32	320 (R)	640	128 (R)	256

Ocular condition	Strains	Ciprofloxacin ≤1, 2, ≥ 4* µg/ml		Ceftazidime ≤8, 16, ≥ 32 µg/ml		Oxacillin ≤ 2, ≥ 4 µg/ml		Gentamicin ≤4, 8, ≥16 µg/ml		Vancomycin ≤2, 4-8, ≥16 µg/ml		Chloramphenicol ≤8, 16, ≥ 32 µg/ml		Azithromycin ≤2, 4 ≥ 8 µg/ml		Polymyxin B ≤2, 4, ≥ 8	
		MIC	MBC	MIC	MBC	MIC	MBC	MBC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Microbial keratitis AUS	M30-01	1 (S)	4	128(R)	256	1 (S)	2	1 (S)	2	1 (S)	2	64 (R)	128	8 (R)	16	128(R)	256
	M36-01	2 (I)	4	128(R)	256	2 (S)	2	2 (S)	2	1 (S)	2	64 (R)	128	128 (R)	256	128 (R)	256
	M43-01	128 (R)	256	128 (R)	256	4 (R)	8	2 (S)	4	1 (S)	2	16 (I)	32	8 (R)	16	64 (R)	128
	M49-02	2 (I)	8	128 (R)	256	2 (S)	4	4 (S)	4	1 (S)	2	128 (R)	256	16 (R)	32	128 (R)	256
	M65-02	1 (S)	4	128 (R)	256	0.5 (S)	1	4 (S)	4	1 (S)	2	64 (R)	64	8 (R)	16	128 (R)	256
	M71-01	4 (R)	16	128 (R)	256	1 (S)	1	1 (S)	2	1 (S)	2	128 (R)	256	128 (R)	256	128 (R)	256
	M90-01	2 (I)	4	64 (R)	128	0.5 (S)	1	1 (S)	2	1 (S)	2	128 (R)	256	128 (R)	256	128 (R)	256
	M91-01	1 (S)	2	128 (R)	256	1 (S)	2	2 (S)	4	2 (S)	2	128(R)	256	16 (R)	16	128 (R)	256
Conjunctivitis USA	84	16 (R)	32	64 (R)	128	8 (R)	16	1 (S)	1	0.5 (S)	1	8 (S)	16	32 (R)	32	8 (R)	64
	85	2 (I)	4	128(R)	256	1 (S)	2	0.5 (S)	0.5	0.5 (S)	1	2 (S)	8	4 (I)	8	4 (I)	16
	86	1 (S)	2	128 (R)	256	0.5 (S)	1	0.25(S)	0.5	0.5 (S)	1	32 (R)	64	128 (R)	256	2 (I)	4
	87	2 (I)	4	128 (R)	256	0.5 (S)	1	0.5 (S)	1	0.5 (S)	1	2 (S)	4	4 (I)	16	2 (I)	4
	88	8 (R)	16	128 (R)	256	1 (S)	2	0.25(S)	1	0.5 (S)	1	2 (S)	4	2 (S)	4	2 (I)	32

	89	1 (S)	2	128 (R)	256	0.5 (S)	1	0.5 (S)	1	1 (S)	2	2 (S)	8	8 (R)	16	8 (R)	16
	90	64 (R)	128	128 (R)	256	0.5 (S)	1	0.5 (S)	1	1 (S)	1	0.5 (S)	1	16 (R)	32	4 (I)	16
	91	1 (S)	2	128 (R)	256	0.5 (S)	1	0.25 (S)	0.5	0.5 (S)	1	1 (S)	2	4 (I)	16	8 (R)	16

Ocular condition	Strains	Ciprofloxacin ≤1, 2, ≥ 4* µg/ml		Ceftazidime ≤8, 16, ≥ 32 µg/ml		Oxacillin ≤ 2, ≥ 4 µg/ml		Gentamicin ≤4, 8, ≥16 µg/ml		Vancomycin ≤2, 4-8, ≥16 µg/ml		Chloramphenicol ≤8, 16, ≥ 32 µg/ml		Azithromycin ≤2, 4 ≥ 8 µg/ml		Polymyxin B ≤2, 4, ≥ 8	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Conjunctivitis USA	**92**	1 (S)	2	4 (S)	16	0.5 (S)	1	0.5 (S)	1	0.5 (S)	1	2 (S)	4	64 (R)	128	4 (I)	16
	93	4 (R)	16	128 (R)	256	1 (S)	2	0.25 (S)	0.5	0.5 (S)	1	2 (S)	4	2 (S)	4	4 (I)	16
	94	8 (R)	16	128 (R)	256	2 (S)	2	1 (S)	1	0.5 (S)	1	0.5 (S)	1	4 (I)	4	4 (I)	16
	95	16 (R)	32	64 (R)	32	0.5 (S)	1	0.25 (S)	0.5	0.5 (S)	1	1 (S)	2	32 (R)	64	4 (I)	16
	96	1 (S)	2	128 (R)	256	1 (S)	2	0.25 (S)	1	0.5 (S)	1	2 (S)	4	4 (I)	16	4 (I)	16
	97	0.25 (S)	0.5	0.5 (S)	1	0.5 (S)	1	0.25 (S)	0.5	0.5 (S)	1	0.5 (S)	1	2 (S)	8	4 (I)	16
	98	0.25 (S)	0.5	128 (R)	256	1 (S)	2	0.25 (S)	0.5	0.5 (S)	0.5	2 (S)	4	1 (S)	4	4 (I)	16
	99	4 (R)	8	128 (R)	256	0.5 (S)	1	0.25 (S)	0.5	0.5 (S)	1	2 (S)	4	4 (I)	16	0.5 (S)	1
	100	0.25 (S)	1	128 (R)	256	0.5 (S)	1	0.25 (S)	0.5	0.5 (S)	1	1 (S)	2	4 (I)	16	8 (R)	16
	101	128 (R)	256	128 (R)	256	64 (R)	128	0.25 (S)	0.5	0.5 (S)	1	2 (S)	2	64 (R)	128	4 (I)	8
	102	32 (R)	64	32 (R)	64	32 (R)	64	1 (S)	2	0.5 (S)	1	1 (S)	2	4 (I)	8	4 (I)	16
	103	32 (R)	64	128 (R)	256	8 (R)	16	0.25 (S)	1	0.5 (S)	0.5	4 (S)	8	32 (R)	64	4 (I)	8

	104	128(R)	256	64 (R)	128	128 (R)	256	0.5 (S)	1	0.5 (S)	1	0.5 (S)	1	64 (R)	128	4 (I)	8
	105	128 (R)	256	32 (R)	64	32 (R)	64	1 (S)	1	1 (S)	1	0.5 (S)	1	64 (R)	128	32 (R)	64

Ocular condition	Strains	Ciprofloxacin ≤1, 2, ≥ 4* µg/ml		Ceftazidime ≤8, 16, ≥ 32 µg/ml		Oxacillin ≤ 2, ≥ 4 µg/ml		Gentamicin ≤4, 8, ≥16 µg/ml		Vancomycin ≤2, 4-8, ≥16 µg/ml		Chloramphenicol ≤8, 16, ≥ 32 µg/ml		Azithromycin ≤2, 4 ≥ 8 µg/ml		Polymyxin B ≤2, 4, ≥ 8	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Conjunctivitis AUS	46	1 (S)	1	4 (S)	8	1 (S)	1	0.5 (S)	1	1 (S)	1	8 (S)	8	4 (I)	8	4 (I)	8
	136	4 (R)	16	16 (R)	32	2 (S)	2	1 (S)	1	0.25 (S)	1	16 (I)	32	8 (R)	32	16 (R)	64
	134	1 (S)	2	32 (R)	64	0.5 (S)	1	0.5 (S)	1	0.5 (S)	0.5	4 (S)	4	8 (R)	16	64 (R)	128
	140	1 (S)	2	64 (R)	128	0.5 (S)	1	0.5 (S)	1	0.5 (S)	1	8 (S)	16	16 (R)	32	128 (R)	16
Non-infectious CIE	12	4 (R)	16	64 (R)	128	1 (S)	2	2 (S)	2	1 (S)	1	8 (S)	16	16 (R)	32	4 (I)	8
	20	1 (S)	2	32 (R)	64	1 (S)	1	1 (S)	2	0.5 (S)	1	32 (R)	64	16 (R)	32	16 (R)	8
	24	0.25 (S)	1	64 (R)	64	0.25 (S)	1	1 (S)	1	0.25 (S)	1	2 (S)	8	4 (I)	16	64 (R)	64
	25	1 (S)	2	8 (S)	32	0.5 (S)	2	1 (S)	2	1 (S)	1	8 (S)	16	8 (R)	32	32 (R)	256
	27	1 (S)	2	64 (R)	128	8 (R)	16	1 (S)	2	1 (S)	2	2 (S)	8	8 (R)	16	64 (R)	16
	28	0.25 (S)	1	4 (S)	8	0.5 (S)	2	0.5 (S)	1	0.5 (S)	2	2 (S)	4	8 (R)	16	4 (I)	16
	32	1 (S)	2	2 (S)	4	0.5 (S)	1	2 (S)	2	0.5 (S)	1	4 (S)	8	2 (S)	4	4 (I)	16
	33	0.5 (S)	1	2 (S)	4	0.5 (S)	1	0.5 (S)	1	0.5 (S)	1	2 (S)	8	4 (I)	16	2 (I)	4
	48	1 (S)	2	16 (R)	32	1 (S)	2	0.5 (S)	1	0.5 (S)	1	16 (R)	32	4 (I)	16	128 (R)	256

	117	8 (R)	16	32 (R)	64	2 (S)	4	0.5 (S)	1	1 (S)	2	8(S)	16	8 (R)	16	128(R)	256

Ocular condition	Strains	Ciprofloxacin ≤1, 2, ≥ 4* µg/ml		Ceftazidime ≤8, 16, ≥ 32 µg/ml		Oxacillin ≤ 2, ≥ 4 µg/ml		Gentamicin ≤4, 8, ≥16 µg/ml		Vancomycin ≤2, 4-8, ≥16 µg/ml		Chloramphenicol ≤8, 16, ≥ 32 µg/ml		Azithromycin ≤2, 4 ≥ 8 µg/ml		Polymyxin B ≤2, 4, ≥ 8	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Non-infectious CIE	**26**	0.5 (S)	1	64 (R)	128	0.25 (S)	0.5	0.5 (S)	1	0.5 (S)	0.5	2 (S)	2	64 (R)	128	8 (R)	16
	29	1 (S)	2	64 (R)	64	0.5 (S)	1	1 (S)	2	0.5 (S)	1	4 (S)	8	16 (R)	64	128 (R)	256
	31	4 (R)	16	16 (R)	32	1 (S)	2	0.5(S)	1	0.5 (S)	1	16 (I)	32	8 (R)	16	128 (R)	256
	41	4 (R)	8	64 (R)	128	1 (S)	2	1 (S)	1	1 (S)	2	2 (S)	4	64 (R)	64	4 (I)	8

*, break points for each antibiotic form CLSI and EUCAST. R= resistant, I = Intermediate, S= susceptible. Conj. = conjunctivitis, MK = microbial keratitis, niCIE = non-infectious corneal infiltrative events. Grey shade indicates resistance.

Multipurpose solution susceptibility

Isolates from contact lens-related niCIE were tested for their susceptibility to MPDS. All MPDS showed good activity against the isolates when used at 100% concentration. After diluting the MPDS, strains were able to grow at different dilutions. Overall, OPTI-FREE PureMoist had the lowest median MIC 5.64% and MBC of 11.36% followed by Renu Advanced Formula (median MIC of 11.36% and MBC of 22.72%). Complete RevitaLens OcuTec and Biotrue had similar median MICs of 22.72% and MBCs of 45.45% (Table 2). There was a significant difference in MIC between OPTI-FREE PureMoist and Biotrue ($p = 0.02$), where strains were more likely to be resistant to Biotrue. One MDR strain (*S aureus* 27) had relatively high MIC and MBC to Biotrue and Renu Advanced Formula of 90.9%, and also moderately high levels for OPTI-FREE PureMoist and Complete RevitaLens Ocutec. The MBCs for all the MPDS were usually twice the MICs.

Table 2. Minimum inhibitory and bactericidal concentrations of MPDS for *S. aureus* niCIE isolates associated with contact lenses.

***S. aureus* Strains**	**OPTI-FREE PureMoist (%)**		**Renu Advanced Formula (%)**		**Complete RevitaLens OcuTec (%)**		**Biotrue (%)**	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
12	2.84	11.36	2.84	5.64	2.84	5.64	11.36	22.72
20	11.36	22.72	11.36	22.72	22.72	22.72	45.45	90.9
24	5.64	11.36	2.84	11.36	45.45	90.9	11.36	22.72
25	1.42	2.84	1.42	5.64	2.84	5.64	5.64	11.36
26	1.42	5.64	1.42	2.84	5.64	11.36	22.72	45.45
27	22.72	22.72	90.9	90.9	22.72	45.45	90.9	90.9
28	11.36	22.72	11.36	22.72	22.72	45.45	45.45	90.9
29	5.64	11.36	22.72	45.45	22.72	45.45	45.45	90.9
31	11.36	22.72	22.72	45.45	22.72	45.45	5.64	11.36
32	5.64	11.36	22.72	45.45	22.72	45.45	22.72	45.45
33	11.36	22.72	22.72	45.45	22.72	45.45	45.45	90.9
41	5.64	11.36	11.36	45.45	11.36	45.45	22.72	45.45
48	2.84	5.64	2.84	5.64	2.84	5.64	5.64	11.36
117	11.36	22.72	5.64	22.72	11.36	11.36	11.36	22.72

Antibiotic and MPDS susceptibility of niCIE strains

Table 3. shows relative susceptibilities of the niCIE strains to antibiotics and MPDS. Bacterial strains can be described as susceptible or resistant to an antibiotic, however there is no such definition for MPDS. A previous study [23] categorized strains with MIC greater than 10% as resistant to MPDS and this classification was used in the current study. There was no concordance between antibiotic and MPDS sensitivity, so antibiotic sensitivity was not a good predictor of resistance to MPDS. One strain (*S. aureus* 27) was resistant to 4/8 antibiotics and all MPDS. Conversely, the strains *S. aureus* 28 and 33 were susceptible to 6/8 antibiotics but were resistant to all MPDS.

Table 3. Relative susceptibilities of contact lens-related niCIE isolates to antibiotics and MPDS.

Strains	**ANTIBIOTICS**	**MPDS**

	CIP	CEFT	OXA	GEN	VAN	CHL	AZI	P-B	OPTI	RENU	REV	BIO
12												
20												
24												
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31												
41												

No shading indicates that strains were susceptible, and grey indicates they were resistant. CIP, Ciprofloxacin; CEFT, Ceftazidime; OXA, Oxacillin; GEN, Gentamicin; VAN, Vancomycin; CHL, Chloramphenicol; AZI, Azithromycin; P-B, Polymyxin B; OPTI, OPTI-FREE PureMoist; RENU, Renu Advanced Formula; REV, Complete RevitaLens OcuTec; BIO, Biotrue.

Discussion

This study reports the *in vitro* susceptibility of ocular strains of *Staphylococcus aureus* from the USA and Australia to commonly used antibiotics and the susceptibility of some strains to contact lens MPDS. Microbial keratitis strains from Australia were more commonly sensitive to fluoroquinolones and oxacillin than strains from the USA. Differences in the antibiotic susceptibility profiles in different geographical populations is not uncommon and may be due to climate [24] or cultural differences [25], [26,], [27], [28]. One study has shown that widespread over-the-counter supply of antibiotics can underpin high resistance [29] and the ability to access antibiotics in such a way differs between countries.

All strains were susceptible to vancomycin 100% and gentamicin 98%. Vancomycin-resistance in systemic infections has been reported, [30] however, no resistance has been reported in ocular isolates [31]. Gentamicin is commonly prescribed in *S. aureus* ocular infections but its susceptibility rates vary [32]. The current results are consistent with other studies from the USA and Australia for *S. aureus* ocular isolates [10],[33],[34],[35]. The antibiotic susceptibility profile in the current study suggests gentamicin to be the best option to treat *S. aureus* ocular infections in both Australia and the USA, and vancomycin to be reserved to treat isolates that are resistant to other antibiotics.

Overall, less than half (46%; 29/63) of all strains in the current study were sensitive to ciprofloxacin. Studies from Australia published between 2014 to 2016 reported 93 to 100% of microbial keratitis isolates were susceptible to ciprofloxacin [36] [37] [38] [39]. In contrast, the current study reports increasing resistance of *S. aureus* strains from Australia to ciprofloxacin (66%). The increasing rate of ciprofloxacin resistance in Australian microbial keratitis strains is of concern, as fluoroquinolones are the first line of treatment for keratitis in Australia [4]. It would be important to explore this in a larger study. Similarly, the rate of resistance of USA ocular *S. aureus* isolates to ciprofloxacin in the current study was higher than in Australia. One possible reason is that in Australia, antibiotic use in animals is restricted compared to other countries including USA [40], which may account for low level of resistance of Australian isolates. It is generally believed that bacteria that infect eye are derived from a general pool of environmental bacteria. Resistant bacteria are transmitted to humans through direct contact with animals [41], through environment [42] and food products [43]. In USA, increasing antibiotic resistance has been attributed to widespread of systemic use, as well as over the counter availability, and in appropriate use for prophylaxis [44]. A large surveillance study from the USA of the antibiotic resistance among ocular isolates between 2009-2016 found approximately 36% of the ocular *S. aureus* isolates were resistant to ciprofloxacin [45]. Increased proportion of MRSA from 8.5% to 27.9%, in *S. aureus* isolates collected between 1990-2001 is reported in USA [46]. MRSA strains are often resistant to fluoroquinolones [45,47,48]. However, in the current study only 7% of MRSA strains from Australia were ciprofloxacin resistant, whereas 78% of MRSA strains from the USA were resistant to ciprofloxacin, which is consistent with a previous report from the USA [45]. The mechanism of resistance of ocular MRSA strains resistant to ciprofloxacin is unclear and requires further study.

In the current study, only 11% of *S. aureus* strains were susceptible to ceftazidime, and all microbial keratitis strains were resistant to this antibiotic. Increasing rate of resistance of *S. aureus* microbial keratitis isolates to first generation cephalosporins (cephalothin) over a period of 15 years has been reported [49]. Ceftazidime is generally reported to be active against *S. aureus* except MRSA strains, but it is less active against *S. aureus* than first and second generation cephalosporins [50]. Resistance to ceftazidime, a third-generation cephalosporin which can be used to treat MRSA, is horizontally acquired due to β –lactamases or altered and over-expression of penicillin binding protein [51]. In the current study the mechanism of resistance may be different depending on the disease or country from which the strains were isolated.

In the present study, chloramphenicol remained a good choice of treatment for conjunctivitis and niCIE caused by *S. aureus* as 96% and 78% of isolates, respectively, were susceptible. Previous reports of Gram-positive bacteria isolated from microbial keratitis isolates have also reported low levels of chloramphenicol resistance in Australian and the USA isolates [52,53]. However, the current study findings of increasing resistance of microbial keratitis strains from Australia (86%) and the USA (45%) are not consistent with these earlier studies and suggest it is a poor choice for treatment for corneal infections. Resistance to chloramphenicol may be inherited [54-56] or acquired [57-59]. The underlying mechanism for the difference in chloramphenicol susceptibility between infectious (MK+ conjunctivitis) and non-infectious ocular conditions requires further investigation.

The majority of *S. aureus* strains in the current study were resistant to azithromycin. Most of the resistant strains were also MRSA, which supports the results of a previous study [10], and most of the strains were resistant to polymyxin B. Polymyxin B is considered a Gram-negative antibiotic that does not diffuse well in medium, and resistance to this antibiotic is characteristic of *Staphylococcus aureus*

[60]. This study supports previous recommendations that Polymyxin B is not a good choice for the treatment of *S. aureus* ocular infections [61].

Only 6% of Australian strains (2/32) were resistant to oxacillin (i.e., could be classified as MRSA), conversely 45% of all the USA strains (14/31) were resistant. In the USA, an increase in the proportion of MRSA among *S. aureus* ocular isolates from 29.5% in 2000 to 41.6% in 2005 has been reported in a national surveillance study (ARMOR) [10]. The high level of MRSA among *S. aureus* isolates is of concern as MRSA is believed to cause more severe disease than methicillin-sensitive *S. aureus* [62]. Further molecular analysis of the geographical variation of MRSA in the USA and Australian microbial keratitis and conjunctivitis strains, and community or hospital acquired MRSA is required.

The study has demonstrated that niCIE strains of *S. aureus* varied in their susceptibilities to MPDSs. The majority of the strains were susceptible to all MPDSs when used at 100% concentrations, indicating good activity of MPDSs. The most effective, OPTI-FREE PureMoist contains two disinfectants, Polyquaternium-1 and Aldox. Polyquaternium-1 showed good activity against *S. aureus* when used alone, as well as Aldox [63]. Renu Advanced was the second most effective MPDS in the current study. It contains three disinfectants, alexidine, PAPB and polyquaternium-1. All of these disinfectants have been reported to be effective against bacteria [63] [64], [65] [66] and, some against their biofilms [67].

Complete RevitaLens, containing contains alexidine and Polyquaternium, the third most effective MPDS against *S. aureus* isolates in the present study, but has been reported to show equal efficacy to OPTI-FREE against *S. aureus* in a previous study [68]. Even though both the disinfectants are effective against *S. aureus* [63] [66], dilution of MPDS decreased its efficacy. Biotrue was the least effective MPDS against *S. aureus* isolates in current study. Biotrue contains only polyaminopropyl biguanide (PAPB, also known as polyhexamethylene biguanide, PHMB). PAPB is active against *S. aureus* [69], but its efficacy is concentration dependant [70]. One study reported a reduced concentration of PAPB (PHMB) after soaking contact lenses in Biotrue, and this lower concentration was associated with its decreased antimicrobial activity against *S. aureus* [70]. The findings of the current study on the most to least active MPDS against *S. aureus* are in general agreement with another study [70].

Resistance to disinfectants can be mediated by the *qac* gene which can be carried on the same transmissible elements as antibiotic resistance genes [71,72]. Whilst possession of *qac* has been associated with resistance to antibiotics [71], there was no clear phenotypic relationship between antibiotic and MPDS resistance observed in the current study. Strains should be examined genotypically for possession of the *qac* gene in future studies.

In this study *S. aureus* isolated from microbial keratitis from the USA were more likely to be MRSA and multidrug resistant compared with Australian microbial keratitis strains. In addition, microbial keratitis strains from the USA and Australia were less susceptible to antibiotics compared to conjunctivitis and non-infectious CIE strains. Exploring genomic resistance mechanisms and possession of virulence traits between infections (MK+ conjunctivitis) and non-infectious ocular conditions from the USA and Australia may help to understand these susceptibility findings. The findings of this study will help to understand the resistance pattern of ocular *S. aureus* isolates from the USA and Australia, which will further inform treatment options.

Materials and methods

Staphylococcus aureus isolates

63 *S. aureus* clinical isolates were evaluated (Table 4). The identity of the strains was confirmed using the automated identification system VITEK 2 for Gram-positive bacteria (BioMérieux, Baulkham Hills, NSW, Australia) according to the manufacturer's instructions.

Table 4. *S. aureus* ocular isolates used in the study.

***S. aureus* isolates**	**Origin**	**Associated Condition**	**Year of isolation**
106	USA	Microbial keratitis (MK)	2004
107			
108			
109			
110			
111			
112			
113			
114			
129	AUS		2006
34			1997
M5-01			2018
M19-01			
M27-01			
M28-01			
M30-01			
M36-01			
M43-01			
M49-02			
M65-02			
M71-01			
M90-01			
M91-01			
84	USA	Conjunctivitis	2004
85			
86			
87			
88			
89			
90			
91			
92			
93			
94			
95			
96			
97			
98			
99			
100			
101			

102			
103			
104			
105			
46	AUS		2006
134			
136			
140			
12	AUS	Contact lens-related non-infectious corneal infiltrative events (niCIE)	1995
20			
24			1996
25			
26			
27			1997
28			
29			
31			
32			
33			
41			1999
48			2001
117			1999

Susceptibility to Antibiotics

The susceptibility of *S. aureus* strains to different antibiotics was assessed according to the standard protocol described by the Clinical and Laboratory Institute [73]. Antibiotics commonly used to treat these ocular conditions in Australia and the USA were selected for the test panel, and antibiotic stock solutions were prepared following the manufacturer's recommendations. Antibiotics were diluted in Mueller-Hinton broth (Becton Dickinson and Company, USA) in sterile 96-well plates to give final concentrations ranging from 5120 μg/ml to 0.25 μg/ml. Bacterial cells at a final concentration of $1x10^5$ CFU/mL were then inoculated into 96 wells plates with different dilutions of antibiotics and incubated at 37ºC for 18-24 hours. Growth turbidity was measured using a spectrophotometer (FLUOstar Omega, BMG LABTECH, Germany) at 660nm. The MIC was taken as the lowest concentration of an antibiotic with no visible growth. For minimum bactericidal concentration (MBC), viable counts were performed by subculturing the MIC and the next two higher dilutions of antibiotic. MBC was the concentration of antibiotic that showed 99.99% bacterial killing [74,75]. The results were interpreted using breakpoints from Clinical and Laboratory Standards Institute [73] and the European Committee on Antimicrobial Susceptibility Testing [76]. Both resistant and intermediate resistant strains were considered resistant for the subsequent analyses.

Susceptibility to multipurpose disinfectant solutions

Susceptibility of the bacterial strains isolated from contact lens-related niCIE to four commercially available MPDSs (Table 5) was assessed. This testing was restricted to these isolates as all other strains were isolated from non-contact lens wearers. The MPDS were OPTI-FREE PureMoist (Alcon, Fort Worth, TX, USA), Complete RevitaLens OcuTec (Abbot Medical Optics, Hangzhou ZJ, China),

and Biotrue and Renu Advanced Formula (Bausch + Lomb, Rochester, NY, USA; Table 5). MPDS susceptibility was tested using previously published methods [23,77]. Strains with MIC of more than 10% MPDS were considered resistant. MBC was the concentration of MPDS that gave 99.99% (3 log units) bacterial killing.

Table 5. Multipurpose disinfecting solutions and their active agents

MPDS	**Manufacturer**	**Disinfectants and their concentrations**
OPTI-FREE® Puremoist®	Alcon, Fort Worth, TX, USA	Polyquaternium-1,10ppm; Aldox, 6ppm
Complete RevitaLens OcuTec (now sold as ACUVUE™ RevitaLens)	Abbot Medical Optics, Hangzhou, ZJ, China (Johnson and Johnson Vision)	Alexidine dihydrochloride, 1.6ppm; polyquaternium-1, 3ppm
Biotrue®	Bausch + Lomb, Rochester, NY, USA	Polyaminopropyl biguanide, 1.3ppm; polyquaternium-1,1ppm
Renu® Advanced Formula		Polyaminopropyl biguanide, 0.5ppm; polyquaternium-1, 1.5ppm; alexidine, 2ppm

Statistical analysis

Differences in the frequency of antibiotic susceptibility between infections (MK+ conjunctivitis) and non-infectious (niCIE) groups from Australia and the USA, and MPDS susceptibility in contact lens related niCIE strains only were compared using Fisher's exact test (GraphPad prism,2019, v8.0.2.263). For all analyses P-value <0.05 was considered statistically significant.

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Conflicts of interest

All the authors declare no conflict of interest.

Authors Contributions

MA: Experimental procedure and writing of manuscript.

AK: Conceptualization of study, manuscript review and editing.

FS: Conceptualization of study, manuscript review and editing.

MW: Conceptualization of study, manuscript review and editing.

All authors have approved the final article.

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