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

Madeeha Afzal, Mark Willcox, Fiona Stapleton, Ajay Kumar Vijay
School of Optometry and Vision Science, UNSW Sydney, Australia

Introduction

Staphylococcus aureus can cause microbial keratitis [1], conjunctivitis [2] and non-infectious contact lens related corneal infiltrative events [3]. *S. aureus* infections can be difficult to treat because of its ability to acquire resistant to multiple antibiotics [4]. Contact lens multipurpose disinfectant solutions (MPDS) are used to disinfect contact lenses when they are not being worn. MPDSs are disinfectant used in contact lens cases in order to avoid contamination and microbial growth, but these disinfectants also lose their efficacy when become contaminated by improper use or poor handling and thus bacteria grow and develop resistance against these disinfectants.

Due to 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.

Materials and Methods

***63 S. aureus* strains:** 23 microbial keratitis [MK], 26 conjunctivitis and 14 strains from non-infectious contact lens-related corneal infiltrative [niCIE] were selected. The minimum inhibitory concentration (MIC) was established for the isolates using the broth microdilution method in 96-well plates to give final concentration ranging from 5120 µg/ml to 0.25 µg/ml. The strains were classified as sensitive or resistant by using CLSI and EUCAST breakpoints.

The antimicrobials tested were four multi-purpose disinfecting solutions and eight antibiotics. All the dilutions for antimicrobials were made in phosphate buffer saline. The bacterial inoculum was prepared in Mueller-Hinton broth.

Statistical analysis: Differences in the frequency of antibiotic susceptibility between infectious and non-infectious groups from Australia and the USA, were compared using Fisher's exact test**.** For statistical analysis, p values of ≤ 0.05 were considered significant, and p values ≤0.1 were considered to show a trend for differences.

Results

Table.1: Frequency of antibiotic susceptibility of *S. aureus* isolates from MK or conjunctivitis from USA and Australia.

Ocular Condition		Antibiotics	USA (% susceptible)	Australia (% susceptible)	P-value
Infectious	MK	Ciprofloxacin	11	57	0.04
		Ceftazidime	0	0	>0.99
		Oxacillin	11	93	0.02
		Gentamicin	89	100	0.46
		Vancomycin	100	100	>0.99
		Chloramphenicol	56	14	0.01
		Azithromycin	0	0	>0.99
		Polymyxin B	0	0	>0.99
	Conjunctivitis	Ciprofloxacin	35	75	0.01
		Ceftazidime	9	25	0.27
		Oxacillin	72	100	>0.99
		Gentamicin	100	100	>0.99
		Vancomycin	100	100	>0.99
		Chloramphenicol	95	75	0.01
		Azithromycin	18	0	>0.99
		Polymyxin B	5	0	>0.99

Table.2: Frequency of antibiotic susceptibility of Australian *S. aureus* isolates from infectious and non-infectious ocular conditions.

Antibiotics	Ocular Condition		P-value
	Infectious (MK+conjunctivitis; % susceptible)	**Non-infectious** Corneal Infiltrative events (% susceptible)	
Ciprofloxacin	61	71	0.71
Ceftazidime	5	28	0.14
Oxacillin	*94*	*93*	>0.99
Gentamicin	98	100	>0.99
Vancomycin	100	100	>0.99
Chloramphenicol	28	78	0.01
Azithromycin	0	7	0.4
Polymyxin B	0	0	>0.99

Fig.1: MIC of *S. aureus* niCIE strains to MPDS

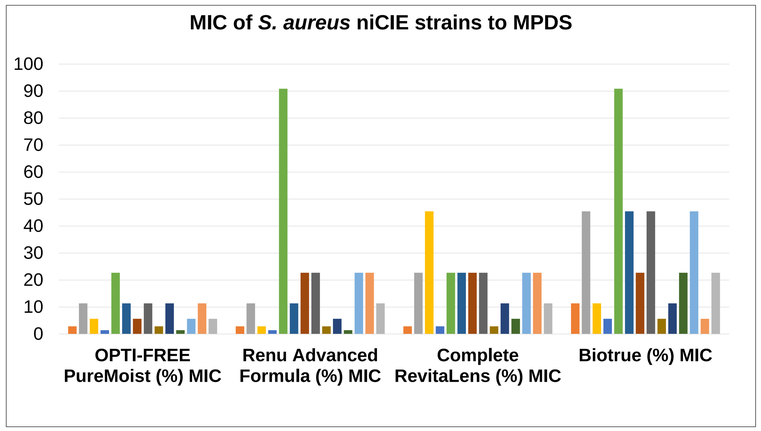

Table.3: Relative susceptibilities of contact lens related niCIE isolates to antibiotics and MPDS

S. aureus Strains	ANTIBIOTICS								MPDS			
	CIP	CEFT	OXA	GEN	VAN	CHL	AZI	P-B	OPTI	RENU	REV	BIO
12	■	■					■	■				■
20		■				■	■	■	■	■		■
24		■					■	■				■
25							■	■				
27	■	■	■				■	■	■	■	■	■
28							■	■	■	■	■	■
32							■	■		■		■
33							■	■	■	■	■	■
48		■				■	■	■				
117	■	■					■	■	■		■	■
26		■					■	■				■
29		■					■	■		■	■	■
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 and Conclusion

- All strains were susceptible to vancomycin (100%) and gentamicin (98%).
- The frequent use of ciprofloxacin in the USA and chloramphenicol in Australia could explain low sensitivity of strains to these antibiotics.
- OPTI-FREE PureMoist was the most effective and Biotrue was the least effective multi-purpose solutions against *S. aureus* niCIE isolates.
- There was no concordance between antibiotic and MPDS sensitivity, so antibiotic sensitivity was not a good predictor of resistance to MPDS.
- Further whole genome sequence will help to better understand resistance mechanisms of *S. aureus* from different ocular conditions.

References

1. O'Callaghan, R.J. The Pathogenesis of Staphylococcus aureus Eye Infections. *Pathogens (Basel, Switzerland)* **2018**, *7*, 9, doi:10.3390/pathogens7010009.
2. Solberg, R.; Meberg, A.; Schøyen, R. [Neonatal conjunctivitis in a nursery and a neonatal unit]. *Tidsskr Nor Laegeforen* **1991**, *111*, 1230-1232.
3. Sweeney, D.F.; Jalbert, I.; Covey, M.; Sankaridurg, P.R.; Vajdic, C.; Holden, B.A.; Sharma, S.; Ramachandran, L.; Willcox, M.D.; Rao, G.N. Clinical characterization of corneal infiltrative events observed with soft contact lens wear. *Cornea* **2003**, *22*, 435-442, doi:10.1097/00003226-200307000-00009.
4. Chambers, H.F.; Deleo, F.R. Waves of resistance: Staphylococcus aureus in the antibiotic era. *Nat Rev Microbiol* **2009**, *7*, 629-641, doi:10.1038/nrmicro2200.
5. Otto, M. Staphylococcus aureus toxins. *Curr Opin Microbiol* **2014**, *17*, 32-37, doi:https://doi.org/10.1016/j.mib.2013.11.004.

The first author is supported by a Tuition Fee Scholarship (TFS) from UNSW

No financial disclosure

Email: m.afzal@unsw.edu.au