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Antibiotic Resistance Determinants among Ocular vs Non-Ocular *Staphylococcus aureus* Clinical Isolates †

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Abstract: Staphylococcus aureus is an important pathogen, and the etiologic agent of more than 70% of ocular and pe-ri-orbital infections causing severe tissue damage, including permanent blindness. Ocular infections may be confounded by antibiotic resistance, and the breadth and nature of resistance among ocular S. aureus isolates is an area of active investigation. Therefore, we harnessed whole genome sequencing of 163 ocular S. aureus isolates from around the world and the CARD antibiotic resistance database to investigate the prevalence of 20 classes of resistance genes. In order to identify emerging circulating resistance determinants that may be of particular importance among ocular S. aureus we utilized a collection of 116 publicly available non-ocular S. aureus genomes as a comparator strain set. Among ocular and non-ocular isolates, antibiotic efflux pumps associated with fluoroquinolone and tetracy-cline resistance were the most prevalent. However, aminoglycoside and macrolide efflux systems, and the tetracycline resistance gene tetM were found more commonly among non-ocular isolates. Moreover, re-sistance determinants for daptomycin, rifampin, and trimethoprim/sulfamethoxazole were only present among non-ocular isolates. In contrast, blaZ-like -lactamases were significantly more prevalent among ocu-lar isolates. Antibiotic resistance prediction software was able to detect significant differences in the antibiotic resistance profiles between ocular and non-ocular S. aureus isolates perhaps reflecting different therapeutic selection pressures in these two groups, and supporting the need for further exploration of S. aureus isolates causing ocular disease.

Keywords: Staphylococcus aureus; whole genome sequencing; ocular infection; antibiotic resistance; Comprehensive Antibiotic Resistance Database

1. Introduction

Infections caused by the Gram-positive pathogen Staphylococcus aureus account for more than 70% of ocu-lar infections worldwide, and can result in rapid tissue damage, including permanent vision loss [1]. Treat-ment for ocular infections is typically empirical, given the risks of permanent damage to vision, and will vary depending on the type of infection. Bacterial keratitis is typically treated with either a fluoroquinolone or a combination of a cephalosporin or vancomycin with an aminoglycoside such as tobramycin [2]. Bacterial conjunctivitis is typically treated with fluoroquinolones or polymyxinB/trimethoprim [3], while endophthal-mitis is a medical emergency and is typically treated with intravenous vancomycin or cefazolin combined with gentamicin or ciprofloxacin [4]. Antibiotic resistance in bacterial pathogens is an area of widespread concern, as treatment failures can result in permanent reduction or complete loss of vision [5]. Despite the impacts of antibiotic resistance on the treatment of ocular infections, prevalence and distribution of re-sistance determinants among S. aureus isolates remains an area of ongoing investigation

2. Methods

Chromosomal DNA was extracted from 163 S. aureus isolates from around the world as part of a larger ef-fort to better understand the virulence determinants of ocular S. aureus infections, and a comparator set of 116 non-ocular S. aureus genomes was obtained from NCBI. Genomes were then run through the Compre-hensive Antibiotic Resistance Database using settings for complete genes and strict hits (≥ 95% identity and coverage) [6]. These results were then collated into a presence or absence profile for 20 putative antibiotic resistance determinants. All data management was performed in Excel, while comparisons were performed by Fisher's Exact Test using the GraphPad QuickCalcs online tool. Differences were considered statistically significant when P-values were ≤ 0.05.

3. Results and Discussion

Of the 20 classifications of antibiotic resistance elements, CARD identified at least 1 putative resistance el-ement for 16 of these elements among the ocular isolates, and 19 of these elements among non-ocular iso-lates (Table 1). No putative genes encoding for vancomycin resistance were detected in ocular or non-ocular isolates, while isolates carrying resistance determinants for daptomycin (N = 3, 2%), rifampin (N = 12, 11%), and trimethoprim/sulfamethoxazole (N = 10, 8%) were present among non-ocular isolates only. Isolates car-rying antibiotic efflux genes were the most frequently identified among both ocular and non-ocular isolates with efflux for fluoroquinolones (N ocular = 151, 92%; N non-ocular = 116, 100%) being the most common (Table 1). No significant differences were observed in the prevalence of efflux genes for fluoroquinolones (Fisher's exact test, P = 0.0917) or tetracyclines (N = 148 (90.80%) and N = 115 (99.14%), P = 0.0929), alt-hough the non-efflux tetracycline resistance gene tetM was significantly more abundant among non-ocular isolates (N = 6 (3.68%), N = 26 (22.41%), P < 0.0001). Aminoglycoside (N = 88 (47.24%), N = 109 (93.97%), P < 0.0001) and macrolide (N = 107 (65.64), N = 115 (99.14%), P < 0.0001) efflux systems were significantly less abundant among ocular isolates, as were enzymes inactivating aminoglycosides (N = 56 (34.36%), N = 46 (39.66%), P < 0.0001). In contrast, enzymes inactivating macrolides were significantly more abundant in ocular isolates (N = 88 (53.99%), N = 35 (30.17%), P < 0.0001) than non-ocular isolates. MRSA isolates were significantly more prevalent among non-ocular isolates (N = 85, 73%) than ocular iso-lates (Fisher's exact test, P < 0.0001) while blaZ-like -lactamases were significantly more prevalent among ocular isolates (N = 115 (71%) vs N = 33 (28%), P < 0.0001).

Table 1. Prevalence of antibiotic resistance determinants among the complete collection of ocular (N = 163) and non-ocular (N = 116) genomes.

Antibiotic	# Ocular	% Ocular	# Non-ocular	% Non-Ocular	P-value
β-lactamase (*blaZ* like)	115	70.55	33	28.45	< 0.0001
MRSA	50	30.67	85	73.28	< 0.0001
aminoglycoside efflux	77	47.24	109	93.97	< 0.0001
aminoglycoside inactivation	56	34.36	46	39.66	< 0.0001
daptomycin	0	0.00	3	2.59	-
fluoroquinolone	151	92.02	116	100.00	0.0917
gyr/*par* mutation	56	34.36	45	38.79	0.4516
fosfomycin	144	88.34	105	90.52	0.6957
fusidic acid	12	7.36	5	4.31	0.3239
lincosamide inactivation	56	34.36	37	31.90	0.7004
macrolide efflux	107	65.64	111	95.69	< 0.0001
macrolide inactivation	88	53.99	35	30.17	< 0.0001
mupirocin	10	6.13	4	3.45	0.4088
phenicol	2	1.23	1	0.86	1.0000
rifampin	0	0.00	12	10.34	-

streptogramin	33	20.25	25	21.55	0.8812
tetM	6	3.68	26	22.41	< 0.0001
tetracycline efflux	148	90.80	115	99.14	0.0929
trimethoprim	0	0.00	10	8.62	-
vancomycin	0	0.00	0	0.00	-

Multilocus sequence types (MLST) 5 and 8 are important circulating sequence types in both ocular and non-ocular isolates, and we compared the prevalence of antibiotic resistance determinants among ocular and non-ocular isolates in each sequence type (Tables 2 and 3). Similar to the larger collection, the prevalence of MLST 5 isolates bearing blaZ like beta-lactamases was significantly higher among ocular isolates than non-ocular isolates (N = 22 (64.71%), N = 4 (25%), P = 0.0145; Table 2) as were isolates bearing macrolide inac-tivation determinants (N = 28 (82.35%), N = 8 (50%), P = 0.0396), while the prevalence of MRSA isolates was significantly lower in ocular isolates than non-ocular isolates (N = 16 (47.06%), N = 13 (81.25%), P = 0.0321; Table 2). Isolates carrying tetM were also significantly less prevalent among MLST 5 ocular isolates than non-ocular isolates (N = 1 (2.94%), N = 4 (25%), P = 0.0313). No mutations conferring resistance to daptomycin, phenicols, or rifampin were detected in either ocular nor non-ocular isolates. No other signifi-cant differences were found in the abundance of antibiotic resistance determinants among MLST 5 isolates (Table 2).

Table 2. Prevalence of antibiotic resistance determinants among MLST 5 ocular (N = 34) and non-ocular (N = 16) genomes.

Antibiotic	**# Ocular**	**% Ocular**	**# Non-ocular**	**% Non-Ocular**	**P-value**
β-lactamase (*blaZ* like)	22	64.71	4	25	0.0145
MRSA	16	47.06	13	81.25	0.0321
aminoglycoside efflux	18	52.94	13	81.25	0.0678
aminoglycoside inactivation	13	38.24	11	68.75	0.0687
daptomycin	0	0.00	0	0	-
fluoroquinolone	31	91.18	16	100	0.5420
gyr/par mutation	30	88.24	13	81.25	0.6657
fosfomycin	1	2.94	0	0	-
fusidic acid	9	26.47	4	25	1.0000
lincosamide inactivation	19	55.88	8	50	0.7666
macrolide efflux	28	82.35	13	81.25	1.0000
macrolide inactivation	28	82.35	8	50	0.0396
mupirocin	4	11.76	1	6.25	1.0000
phenicol	0	0.00	0	0	-
rifampin	0	0.00	0	0	-
streptogramin	13	38.24	3	18.75	0.2084
tetM	1	2.94	4	25	0.0313
tetracycline efflux	31	91.18	16	100	0.5420
trimethoprim	0	0.00	1	6.25	-
vancomycin	0	0.00	0	0	-

Among MLST 8 isolates, blaZ like β-lactamases were significantly more abundant among ocular isolates than non-ocular isolates (N = 16 (66.67%, N = 8 (21.62%), P = 0.0010), however, contrary to MLST 5 and the larger collection, no significant differences in the prevalence of MRSA isolates were observed (N = 10 (41.67%), N = 21 (56.76%), P = 0.3004). MLST 8 isolates followed a similar trend to the larger collection with aminoglycoside efflux genes being less prevalent among ocular isolates

than non-ocular isolates (N = 10 (41.67%), N = 35 (94.59%), P = 0.0099), and macrolide inactivation determinants being more abundant in ocular than non-ocular isolates (N = 16 (66.67%), N = 10 (27.03%), P = 0.0034). No other significant differ-ences were found among the other antibiotic resistance determinants in MLST 8.

Table 3. Prevalence of antibiotic resistance determinants among MLST 8 ocular (N = 24) and non-ocular (N = 37) genomes.

Antibiotic	# Ocular	% Ocular	# Non-ocular	% Non-Ocular	P-value
β-lactamase (*blaZ* like)	16	66.67	8	21.62	0.0010
MRSA	10	41.67	21	56.76	0.3004
aminoglycoside efflux	16	66.67	35	94.59	0.0099
aminoglycoside inactivation	11	45.83	10	27.03	0.1711
daptomycin	0	0.00	2	5.41	-
fluoroquinolone	22	91.67	37	100.00	0.1508
gyr/par mutation	11	45.83	3	27.03	0.0012
fosfomycin	24	100.00	31	83.78	0.0726
fusidic acid	3	12.50	1	2.70	0.2904
lincosamide inactivation	11	45.83	10	27.03	0.1711
macrolide efflux	19	79.17	35	94.59	0.1007
macrolide inactivation	16	66.67	10	27.03	0.0034
mupirocin	2	8.33	0	0.00	-
phenicol	0	0.00	0	0.00	-
rifampin	0	0.00	2	5.41	-
streptogramin	6	25.00	10	27.03	1.0000
tetM	0	0.00	2	5.41	-
tetracycline efflux	22	91.67	35	94.59	1.0000
trimethoprim	0	0.00	6	16.22	-
vancomycin	0	0.00	0	0.00	-

We previously investigated the antibiotic susceptibility profiles of the 163 ocular isolates to erythromycin (69% resistance), levofloxacin (40%), moxifloxacin (33%), polymyxin B/trimethoprim (6.7%), rifampin (3%), tobramycin (17%), trimethoprim (18%) and vancomycin (0%) by minimum inhibitory concentration assay [7]. CARD overestimated the resistance of the fluoroquinolones when assessed by the presence of efflux (99% in silico vs ~40% in vivo) but was much closer when considering mutations conferring resistance (34.6% vs ~40%). In silico prediction similarly overestimated resistance against aminoglycoside antibiotics due to both efflux (47% vs 17%) and inactivation (34% vs 17%). In silico prediction underestimated the prevalence of macrolide resistance by efflux (66% vs 69%) and inactivation (56% vs 69%) rifampin resistance (0% vs 3%) and trimethoprim resistance (0% vs 18%).

4. Conclusions

The antibiotic resistance profiles of ocular and non-ocular isolates were significantly different for 10 of the 20 antibiotic resistance determinants (Table 1). Within the two most abundant MLSTs, there were fewer significant differences overall, however while the abundance of MRSA was significantly greater in non-ocular isolates in the larger collection, within MLST 8, there was no significant difference (Tables 2 and 3). While fluoroquinolones are frequently utilized as front line empirical treatment, no significant differences in mutations within gyrAB/parCE or fluoroquinolone

associated efflux pumps were found between ocular and non-ocular isolates. The increased prevalence of macrolide resistance in ocular compared to non-ocular iso-lates is not surprising, as macrolide antibiotics such as erythromycin, are widely used globally in the treat-ment of conjunctivitis.

In summary, antibiotic resistance prediction software was able to detect significant differences in the antibi-otic resistance profiles between ocular and non-ocular S. aureus isolates, and revealed that overall, antibiotic resistance is lower among ocular isolates than non-ocular isolates which may reflect different therapeutic se-lection pressures in these two groups. A comparison of previously reported in vitro results [7] with the re-sults from the CARD database suggests that there are important differences between predicted resistance elements and functional antibiotic resistance in vitro that should be considered. These results highlight the need for further exploration of antibiotic resistance among S. aureus isolates causing ocular disease.

Conflicts of Interest: The author declares no conflict of interest.

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