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Original Article

Bacterial Ocular Surveillance System (BOSS) Sydney, Australia 2017-2018

Stephanie L. Watson, Barrie J Gatus, Maria Cabrera-Aguas, Benjamin H Armstrong, CR Robert George, Pauline Khoo, Monica M Lahra

Abstract

This study investigated antimicrobial resistance (AMR) profiles from a cohort of patients with bacterial keratitis treated at Sydney Eye Hospital, 1 January 2017 – 31 December 2018. These AMR profiles were analysed in the context of the current Australian empiric regimens for topical therapy: ciprofloxacin/ofloxacin monotherapy versus combination therapy of cefalotin/cephazolin plus gentamicin. At our Centre, combinations of (i) chloramphenicol plus gentamicin and (ii) chloramphenicol plus ciprofloxacin are alternatively used, so were also analysed.

Three hundred and seventy-four isolates were cultured prospectively: 280/374 (75%) were gram positive, and 94/374 (25%) were gram negative. Coagulase-negative staphylococci comprised 173/374 (46%). Isolates included *Staphylococcus aureus* (n = 43/374) 11%; *Streptococcus pneumoniae* (n = 14/374) 3.7%; and *Pseudomonas aeruginosa* (n = 50/374) 13%.

Statistical comparison was performed. There was no significant difference between cover provided either of the current Australian recommendations: ciprofloxacin/ofloxacin vs cefalotin/cephazolin plus gentamicin (5.3% vs 4.8%, respectively; $p = 0.655$). However, the combination of chloramphenicol plus an anti-pseudomonal agent (ciprofloxacin/ofloxacin or gentamicin) had significantly improved cover.

Chloramphenicol plus gentamicin was superior to ciprofloxacin/ofloxacin (1.9% vs 5.3% resistance respectively; $p = 0.007$), and cefalotin/cephazolin plus gentamicin (1.9% vs 4.8%; $p = 0.005$). Chloramphenicol plus ciprofloxacin was superior to ciprofloxacin/ofloxacin monotherapy (1.3% vs 5.3%; $p \leq 0.001$), and to cefalotin/cephazolin plus gentamicin (1.3% vs 4.8%; $p = 0.003$). Chloramphenicol plus gentamicin versus chloramphenicol plus ciprofloxacin/ofloxacin were equivalent ($p = 0.48$).

There was no demonstrated *in vitro* superiority of either the current empiric antibiotic regimens. For our setting, for bacterial keratitis, chloramphenicol in combination offered superior *in vitro* cover. Broadened surveillance for ocular AMR is urgently needed across jurisdictions.

Keywords: Antibiotic resistance, bacterial keratitis, corneal scrape, surveillance

Introduction

Antimicrobial resistance (AMR) is a global health threat recognised across patient populations as having a significant potential impact on treatment outcomes.[1,2] In the USA, resistant infections cause about 23,000 deaths and more than two million illnesses annually with indirect societal costs of US$35 billion. Similarly, 25,000 deaths per year related to resistant infections occur in Europe.[1] The Organisation for Economic Co-operation and Development (OECD) estimate an average of 290 deaths each year in Australia are caused by eight resistant bacteria.[3,4] By 2050, approximately 10,430 people will die due to AMR and the health costs will reach $370 million in Australia[4]. Surveillance programs of AMR are recommended by the World Health

Organization Global Action Plan to underpin disease prevention and control strategies.[5] These strategies include evidence-based antibiotic prescribing guidelines, informed with local data, as highlighted in the medical literature.[1,6–9]

Bacterial keratitis (BK) is an ophthalmic emergency requiring immediate and effective antibiotic treatment as it can progress rapidly, causing visual impairment and, potentially, blindness.[10–13] There are significant collateral costs, including a reduced quality of life for the individual, and an increased health-system burden.[10–12,14,15] In the elderly, loss of an eye and blindness have been reported in 10% and 40% of patients, respectively,[16] and in children, permanent visual loss due to amblyopia is a complication.[17] Thus, there is a continued need to undertake AMR surveillance in order to determine the suitability of empiric antibiotic therapy for BK, given the ever-changing challenge of organisms becoming resistant to antibiotics.

In 2016, a keratitis antimicrobial resistance surveillance programme was established at The Sydney Eye Hospital.[18] This report examined the types, frequency of isolation, and the antibiotic resistance profiles of bacteria isolated from corneal scapes of patients where bacterial keratitis was clinically apparent.[18] This was the first comprehensive study of ocular AMR in Australia. The Sydney Eye Hospital, established in 1882, is the largest quaternary opthalmic referral hospital in the southern hemisphere, providing surgical and medical care for patients with corneal, vitreo-retinal, glaucoma, oculo-plastic, uveitis, and oculo-oncology conditions.

Our report of 2016[18] and studies elsewhere[19–22] demonstrate that gram-positive bacteria including coagulase-negative *Staphylococcus* spp., *Staphylococcus aureus* and *Streptococcus pneumoniae* comprise the majority of organisms isolated from the cornea of patients with bacterial keratitis. However, infection with *Pseudomonas aeruginosa* is a major concern, particularly in people wearing contact lenses; thus, empiric, topical antibiotic therapy must include an antibiotic effective both against commonly-isolated gram-positive bacteria and *Pseudomonas aeruginosa*.

The purpose of the present study was to expand and update the information given in our inaugural report of 2016; and to identify the types and prevalence of the different types of bacteria isolated from corneal scrapes of patients with bacterial keratitis at the Sydney Eye Hospital during the period 2017–2018. In addition, the antibiotic susceptibilities of organisms were examined and analysed statistically in order to assess the appropriateness of the guidelines used for the empiric antibiotic treatment of bacterial keratitis in Australia: *Therapeutic Guidelines – Antibiotic, Version 16, 2019*.[23]

The current guidelines in Australia recommend empiric fluoroquinolone monotherapy (0.3% ciprofloxacin or 0.3% ofloxacin) or fortified combination therapy with 5% cephazolin plus 0.9% gentamicin.[22] The combinations of chloramphenicol 0.5% plus gentamicin 0.9%, or chloramphenicol 0.5% plus ciprofloxacin 0.3%, are used on occasion at our Centre as empiric therapy for BK and were therefore included in the analysis. Resistance to chloramphenicol is of additional interest given the drug was made available in Australia over the counter in 2010.[24]

Methods

We conducted a review of the microbiology results of the cohort of patients who presented with clinical bacterial keratitis to Sydney Eye Hospital during the period January 1, 2017 to December 31 2018. Corneal scrape specimens were taken in accord with local protocols from patients who had a clinical diagnosis of keratitis at presentation to the Sydney Eye Hospital.[25] Ethics approval for this study was granted by the Sydney Local Health District Human Research Ethics Committee (approval number: 2020/ETH00457).

The microbiological methods have been previously described in our inaugural report of 2016.[18] Briefly, corneal scapes were inoculated onto agar

media or onto the same media from an enrichment medium. Identification of organisms was performed using matrix assisted laser desorption ionisation–time of flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics® Germany). Antibiotic susceptibility testing was performed via the calibrated dichotomous sensitivity (CDS) agar disc diffusion method.[26]

A statistical comparative analysis of antibiotic resistance to the following regimens was performed: ciprofloxacin/ofloxacin monotherapy, combination therapy with cefalotin/cephazolin plus gentamicin; combination therapy with chloramphenicol plus gentamicin, and combination therapy with ciprofloxacin/ofloxacin and chloramphenicol. These comparisons were made using McNemar's test, with data analysed using Jamovi version 1.2.19.

Results

Bacteria isolated

Three hundred and seventy-four bacterial isolates were cultured prospectively, from 297/471 inoculated plates (a 63% positive culture rate). Of these, 280/374 (75%) were gram positive, as shown in Table 1; and 94/374 (25%) were gram negative, as shown in Table 2. Coagulase-negative staphylococci were isolated most frequently in 173/374 cases (46%). *Staphylococcus aureus* (n = 43/374) comprised 11%; *Streptococcus pneumoniae* (n = 14/374) 3.7%; and *Pseudomonas aeruginosa* (n = 50/374) 13% of the total, as shown in Table 3.

Antibiotic resistance to ciprofloxacin/ofloxacin

There were 20/374 isolates resistant to ciprofloxacin/ofloxacin, giving an overall rate of resistance, as shown in Table 4, of 5.3% with a 95% confidence interval (95% CI) of 3.1–7.6%. With regards to ciprofloxacin/ofloxacin resistance by organisms isolated: for coagulase-negative staphylococci, 10/173 (6%) were resistant; 7/43 (16%) *Staphylococcus aureus* were resistant; 2/12 (17%) *Corynebacterium* spp. were resistant, and 1/9 (11%) *Serratia marcescens* was resistant. No *Pseudomonas aeruginosa* (0/50) isolate was resistant.

Antibiotic resistance to cefalotin/cephazolin

There were 113/374 organisms resistant to cefalotin/cephazolin, giving an overall rate of resistance of 30% as shown in Table 5. For cefalotin/cephazolin, 33/173 coagulase-negative staphylococci (19%) were resistant; and for *Staphylococcus aureus*, 6/43 (14%) were resistant. No *Streptococcus pneumoniae* was resistant to cefalotin/cephazolin (0/14). *Pseudomonas aeruginosa* is intrinsically resistant to cefalotin/cephazolin.

Antibiotic resistance to gentamicin

There were 335 of 374 organisms tested for gentamicin susceptibility. Of these, 44/335 (13%) were resistant, as shown in Table 6. *Moraxella* spp. (n = 24), *Corynebacterium* spp. (n = 12) and *Haemophilus influenzae* (n = 3) were not tested, as calibrations for these organisms against gentamicin are not provided in the CDS method. Of the coagulase-negative staphylococci isolated, there were 14/173 (8.1%) that were resistant to gentamicin, as were 4/43 (9.3%) of *Staphylococcus aureus*. *Streptococcus* species are considered resistant to gentamicin monotherapy. No *Pseudomonas aeruginosa* isolated (0/50) was resistant to gentamicin.

Antibiotic resistance to chloramphenicol

All isolates were tested against chloramphenicol and the proportion resistant was 21% (79/374), as shown in Table 7. Of the coagulase-negative staphylococci isolates, 21/173 (12%) were resistant to chloramphenicol, as were 2/43 (5%) of the *Staphylococcus aureus* isolated. Of the *Streptococcus pneumoniae* isolated, one (1/14; 7%) was resistant to chloramphenicol. *Pseudomonas aeruginosa* has intrinsic resistance to chloramphenicol.

Table 1. The types, numbers (n) and percentages (%) of gram-positive organisms isolated from corneal scrapes during the period 2017–2018.

Organism	n	%
Coagulase-negative staphylococci	173	62
Staphylococcus aureus[a]	43	15
Streptococcus pneumoniae	14	5
Corynebacterium spp.	12	4
Micrococcus luteus	10	4
Streptococcus mitis/oralis group	8	3
Bacillus spp.	6	2
Rothia spp.	3	1
Streptococcus gordonii	2	1
Other[b]	9	3
Total	280	100

a Including methicillin-resistant *Staphylococcus aureus*.

b Other: *Abiotrophia defectiva* (1), *Clostridium sporogenes* (1), *Lysinibacillus sphaericus* (1), *Propionibacterium spp*. (1), *Enterococcus faecalis* (1), *Brevibacillus borstelensis* (1), *Paenibacillus spp*. (1), *Streptococcus sanguinis* (1), *Streptococcus dysgalactiae* (1).

Table 2. The numbers (n) and percentages (%) of gram-negative organisms isolated from corneal scrapes during the period 2017–2018.

Organism	n	%
Pseudomonas aeruginosa	50	53
Moraxella spp.	24	26
Serratia marcescens	9	10
Haemophilus influenzae	3	3
Other[a]	8	9
Total	94	100

a Other: *Acinetobacter* spp. (1), *Achromobacter xylosoxidans* (1), *Enterobacter cloacae* complex (1), *Klebsiella oxytoca* (1), *Roseomonas mucosa* (2), *Citrobacter koseri* (1), *Proteus mirabilis* (1).

Table 3. Frequency (n) and percentage (%) of the total number of organisms isolated from corneal scrapes during the period 2017–2018.

Organism	n	%
Coagulase-negative staphylococci	173	46
Pseudomonas aeruginosa	50	13
Staphylococcus aureus[a]	43	12
Moraxella spp.	24	6
Streptococcus pneumoniae	14	4
Corynebacterium spp.	12	3
Micrococcus luteus	10	3
Serratia marcescens	9	2
Other gram-positive[b]	9	2
Streptococcus mitis/oralis group	8	2
Other gram-negative[c]	8	2
Bacillus spp.	6	2
Rothia spp.	3	1
Haemophilus influenzae	3	1
Streptococcus gordonii	2	1
Total	**374**	**100**

a Including methicillin-resistant *Staphylococcus aureus*.

b Other gram-positive: *Abiotrophia defectiva* (1), *Clostridium sporogenes* (1), *Lysinibacillus sphaericus* (1), *Propionibacterium* spp. (1), *Enterococcus faecalis* (1), *Brevibacillus borstelensis* (1), *Paenibacillus* spp. (1), *Streptococcus sanguinis* (1), *Streptococcus dysgalactiae* (1).

c Other gram-negative: *Acinetobacter* spp. (1), *Achromobacter xylosoxidans* (1), *Enterobacter cloacae* complex (1), *Klebsiella oxytoca* (1), *Roseomonas mucosa* (2), *Citrobacter koseri* (1), *Proteus mirabilis* (1).

Analysis of *in vitro* resistance to combination therapy

Combined antibiotic resistance to cefalotin/cephazolin plus gentamicin

There were 39 isolates (24 *Moraxella* spp., 12 *Corynebacterium* spp. and three *Haemophilus influenzae*) that were not able to be tested for gentamicin susceptibility. All *Moraxella* spp. and *Corynebacterium* spp., plus 2/3 isolates of *Haemophilis influenzae*, were susceptible to cefalotin/cephazolin, and therefore *in vitro* susceptibility to the combined regimen of cefalotin/cephazolin and gentamicin was determined according to tested susceptibility for cefalotin/cephazolin. A single isolate of *Haemophilus influenzae* was resistant to cefalotin/cephazolin, and therefore susceptibility to the combined regimen was unable to be confirmed; this isolate was included in the analysis as likely resistant to combined cefalotin/cephazolin plus gentamicin. For the staphylococci, 12/173 (7%) coagulase-negative staphylococci and 3/43 (7%) *Staphylococcus aureus* were resistant to both agents. No *Streptococcus pneumoniae* isolates (0/14) and no (0/50) *Pseudomonas aeruginosa* were resistant to both agents.

Overall, resistance to the combination of cefalotin/cephazolin plus gentamicin, where both agents were tested, or resistance to cefalotin/cephazolin when gentamicin was not tested, was 4.8% (18/374; 95% CI: 2.6–7.0%). These data are shown in Table 8.

Combined antibiotic resistance to chloramphenicol plus gentamicin

There were 7/374 isolates resistant to the combination of chloramphenicol plus gentamicin giving an overall rate of resistance of 1.9% (95% CI: 0.5–3.2%). Of the coagulase-negative staphylococci, 5/173 (2.9%) were resistant. All isolates of *Staphylococcus aureus* were susceptible (n = 43). A single isolate (1/14; 7.1%) of *Streptococcus pneumoniae* was resistant. All isolates of *Pseudomonas aeruginosa* were susceptible (n = 50). There were 39 isolates (24 *Moraxella* spp., 12 *Corynebacterium* spp. and three *Haemophilus influenzae*) that were not able to be tested for gentamicin susceptibility; however, all were susceptible to chloramphenicol. These data are shown in Table 9 below.

Combined antibiotic resistance to chloramphenicol plus ciprofloxacin/ofloxacin

The data for combined susceptibility to chloramphenicol plus ciprofloxacin/ofloxacin are shown in Table 10. There were 5/374 isolates resistant to the combination of chloramphenicol plus ciprofloxacin, giving an overall rate of resistance of 1.3% (95% CI: 0.2–2.5%). Of the coagulase-negative staphylococci, 3/173 (1.7%) were resistant. All isolates of *Staphylococcus aureus* were susceptible (n = 43). A single isolate each of *Corynebacterium spp.* (1/12; 8.3%), and *Serratia marcescens* (1/9; 11%), were resistant. All isolates of *Pseudomonas aeruginosa* were susceptible (n = 50).

Statistical analysis of differences in coverage of the antibiotic susceptibility between antibiotic combinations

Ciprofloxacin/ofloxacin versus cefalotin/cephazolin plus gentamicin

As displayed in Tables 4 and 8, 20/374 isolates (5.3%) were resistant to ciprofloxacin/ofloxacin, and 18/374 (4.8%) to combined cefalotin/cephazolin plus gentamicin. A single isolate of *Haemophilus influenzae* was resistant to cefalotin/cephazolin, and unable to be tested against gentamicin given the lack of available calibrations. As *in vitro* susceptibility was unable to be confirmed, this isolate was included in the analysis as resistant to the combination of cefalotin/cephazolin and gentamicin. Across all 374 isolates tested, nine isolates were concurrently resistant to both regimens (six isolates of coagulase-negative staphylococci and three isolates of *Staphylococcus aureus*). When the difference in coverage of the two regimens was examined, there was no significant difference detected ($\chi^2 = 0.20$; degrees of freedom (df) = 1; $p = 0.655$).

Table 4. The total number of organisms isolated from corneal scrapes during the period 2017–2018 and the number (n) and percentage (%) resistant to ciprofloxacin/ofloxacin.

Organism	Total	Resistant	
		n	%
Coagulase-negative staphylococci	173	10	6
Pseudomonas aeruginosa	50	0	0
Staphylococcus aureus[a]	43	7	16
Moraxella spp.	24	0	0
Streptococcus pneumoniae	14	0	0
Corynebacterium spp.	12	2	17
Micrococcus luteus	10	0	0
Serratia marcescens	9	1	11
Other gram-positive[b]	9	0	0
Streptococcus mitis/oralis group	8	0	0
Other gram-negative[c]	8	0	0
Bacillus spp.	6	0	0
Rothia spp.	3	0	0
Haemophilus influenzae	3	0	0
Streptococcus gordonii	2	0	0
Total	**374**	**20**	**5.3**

a Including methicillin-resistant *Staphylococcus aureus*.

b Other gram-positive: *Abiotrophia defectiva* (1), *Clostridium sporogenes* (1), *Lysinibacillus sphaericus* (1), *Propionibacterium* spp. (1), *Enterococcus faecalis* (1), *Brevibacillus borstelensis* (1), *Paenibacillus* spp. (1), *Streptococcus sanguinis* (1), *Streptococcus dysgalactiae* (1).

c Other gram-negative: *Acinetobacter* spp. (1), *Achromobacter xylosoxidans* (1), *Enterobacter cloacae* complex (1), *Klebsiella oxytoca* (1), *Roseomonas mucosa* (2), *Citrobacter koseri* (1), *Proteus mirabilis* (1).

Table 5. The total number of organisms isolated from corneal scrapes during the period 2017–2018 and the number (n) and the percentage (%) resistant to cefalotin/cephazolin.

Organism	Total	Resistant	
		(n)	(%)
Coagulase-negative staphylococci	173	33	19
Pseudomonas aeruginosa (IR)[a]	50	50	100
Staphylococcus aureus[b]	43	6	14
Moraxella spp.	24	0	0
Streptococcus pneumoniae	14	0	0
Corynebacterium spp.	12	0	0
Micrococcus luteus	10	0	0
Serratia marcescens (IR)[a]	9	9	100
Other gram-positive[c]	9	0	0
Streptococcus mitis/oralis group	8	2	25
Other gram-negative[d,e]	8	8	100
Bacillus spp.	6	4	66
Rothia spp.	3	0	0
Haemophilus influenzae	3	1	33
Streptococcus gordonii	2	0	0
Total	**374**	**113**	**30**

a IR: Intrinsic resistance, denotes organisms intrinsically resistant to cefalotin/cephazolin.

b Including methicillin-resistant *Staphylococcus aureus*.

c Other gram-positive: *Abiotrophia defectiva* (1), *Clostridium sporogenes* (1), *Lysinibacillus sphaericus* (1), *Propionibacterium* spp. (1), *Enterococcus faecalis* (1), *Brevibacillus borstelensis* (1), *Paenibacillus* spp. (1), *Streptococcus sanguinis* (1), *Streptococcus dysgalactiae* (1).

d Other gram-negative: *Klebsiella oxytoca* (1), *Citrobacter koseri* (1), *Proteus mirabilis* (1) were tested and displayed acquired resistance to cefalotin/cephazolin.

e *Acinetobacter* spp. (1), *Achromobacter xylosoxidans* (1), *Enterobacter cloacae* complex (1), *Roseomonas mucosa* (2) are considered intrinsically resistant.

Table 6. The total number of organisms isolated from corneal scrapes and tested against gentamicin during the period 2017–2018 and the number (n) and percentage (%) resistant.

Organism	Total	Resistant	
		(n)	(%)
Coagulase-negative staphylococci	173	14	8
Pseudomonas aeruginosa	50	0	0
Staphylococcus aureus[a]	43	4	9
Streptococcus pneumoniae	14	14	100
Micrococcus luteus	10	0	0
Serratia marcescens	9	0	0
Other gram-positive[b]	9	1	12
Streptococcus mitis/oralis group	8	8	100
Other gram-negative[c]	8	1	12
Bacillus spp.	6	0	0
Rothia spp.	3	0	0
Streptococcus gordonii	2	2	100
Total	**335**	**44**	**13**

a Including methicillin-resistant *Staphylococcus aureus*.

b Other gram-positive: *Abiotrophia defectiva* (1), *Clostridium sporogenes* (1), *Lysinibacillus sphaericus* (1), *Propionibacterium* spp. (1), *Enterococcus faecalis* (1), *Brevibacillus borstelensis* (1), *Paenibacillus* spp. (1), *Streptococcus sanguinis* (1), *Streptococcus dysgalactiae* (1).

c Other gram-negative: *Acinetobacter* spp. (1), *Achromobacter xylosoxidans* (1), *Enterobacter cloacae* complex (1), *Klebsiella oxytoca* (1), *Roseomonas mucosa* (2), *Citrobacter koseri* (1), *Proteus mirabilis* (1).

Table 7. The total number of organisms isolated from corneal scrapes during the period 2017–2018 and the number (n) and percentage (%) resistant to chloramphenicol.

Organism	Total	Resistant	
		(n)	(%)
Coagulase-negative staphylococci	173	21	12
Pseudomonas aeruginosa (IR)[a]	50	50	100
Staphylococcus aureus[b]	43	2	5
Moraxella spp.	24	0	0
Streptococcus pneumoniae	14	1	7
Corynebacterium spp.	12	2	2
Micrococcus luteus	10	0	0
Serratia marcescens	9	1	11
Other gram-positive[c]	9	1	11
Streptococcus mitis/oralis group	8	0	0
Other gram-negative[d]	8	1	13
Bacillus spp.	6	0	0
Rothia spp.	3	0	0
Haemophilus influenzae	3	0	0
Streptococcus gordonii	2	0	0
Total	**374**	**79**	**21**

a IR: Intrinsic resistance.

b Including methicillin-resistant *Staphylococcus aureus*.

c Other gram-positive: *Abiotrophia defectiva* (1), *Clostridium sporogenes* (1), *Lysinibacillus sphaericus* (1), *Propionibacterium* spp. (1), *Enterococcus faecalis* (1), *Brevibacillus borstelensis* (1), *Paenibacillus* spp. (1), *Streptococcus sanguinis* (1), *Streptococcus dysgalactiae* (1).

d Other gram-negative: *Acinetobacter* spp. (1), *Achromobacter xylosoxidans* (1), *Enterobacter cloacae* complex (1), *Klebsiella oxytoca* (1), *Roseomonas mucosa* (2), *Citrobacter koseri* (1), *Proteus mirabilis* (1).

Chloramphenicol plus gentamicin versus cefalotin/cephazolin plus gentamicin

Of the isolates tested, 18/374 (4.8%) were resistant to combined cefalotin/cephazolin plus gentamicin, and 7/374 isolates (1.9%) were resistant to chloramphenicol plus gentamicin, as shown in Tables 8 and 9. A single isolate of *Haemophilus influenzae* was resistant to cefalotin/cephazolin, and unable to be tested against gentamicin. As *in vitro* susceptibility was unable to be confirmed, this isolate was included in the analysis as resistant to the combination of cefalotin/cephazolin and gentamicin. Five isolates (all coagulase-negative staphylococci) were resistant to all three agents. Statistically, the combination of chloramphenicol plus gentamicin had significantly better coverage across all isolates than the combination of cefalotin/cephazolin plus gentamicin ($\chi^2 = 8.07$; df = 1; $p = 0.005$).

Ciprofloxacin/ofloxacin versus chloramphenicol plus gentamicin

As shown in Tables 4 and 9, 20/374 isolates (5.3%) were resistant to ciprofloxacin/ofloxacin, and 7/374 (1.9%) to combined chloramphenicol plus gentamicin. Of these, only 2/374 isolates were concurrently resistant to both regimens (both coagulase-negative *Staphylococcus* spp.). Statistically, the combination of chloramphenicol plus gentamicin had significantly better coverage across all isolates than ciprofloxacin/ofloxacin ($\chi^2 = 7.35$; df = 1; $p = 0.007$).

Ciprofloxacin/ofloxacin monotherapy versus ciprofloxacin/ofloxacin plus chloramphenicol

As displayed in Tables 4 and 10, the addition of chloramphenicol to ciprofloxacin/ofloxacin reduced *in vitro* predicted treatment failure from 20/374 (5.3%) to 5/374 isolates (1.3%). *In vitro* resistance to the combination of chloramphenicol and ciprofloxacin/ofloxacin was detected in three isolates of coagulase-negative *Staphylococcus* spp., one *Serratia marcescens* and one *Corynebacterium* spp. The combination of ciprofloxacin/ofloxacin and chloramphenicol offered statistically significantly improved cover over ciprofloxacin/ofloxacin monotherapy ($\chi^2 = 16.00$; df = 1; $p \leq 0.001$).

Cefalotin/cephazolin plus gentamicin versus ciprofloxacin/ofloxacin plus chloramphenicol

As displayed in Tables 8 and 10, 18/374 (4.8%) isolates were resistant to combined cefalotin/cephazolin plus gentamicin, and 5/374 (1.3%) to combined chloramphenicol plus ciprofloxacin/ofloxacin. Of these, only 2/374 isolates were concurrently resistant to both regimens (both coagulase-negative *Staphylococcus* spp.). The combination of ciprofloxacin/ofloxacin and chloramphenicol offered statistically significantly improved cover over cephazolin/cefalotin plus gentamicin ($\chi^2 = 8.89$; df = 1; $p = 0.003$).

Chloramphenicol plus gentamicin versus ciprofloxacin/ofloxacin plus chloramphenicol

As displayed in Tables 9 and 10, 7/374 (1.9%) isolates were resistant to chloramphenicol plus gentamicin, and 5/374 (1.3%) to combined chloramphenicol plus ciprofloxacin/ofloxacin. Of these, only 2/374 isolates were concurrently resistant to both regimens (both coagulase-negative *Staphylococcus spp*). When the difference in coverage of these two regimens was examined, there was no significant difference detected ($\chi^2 = 0.50$; df = 1; $p = 0.480$).

Table 8. Combined resistance to cefalotin/cephazolin plus gentamicin: the total number of organisms isolated from corneal scrapes during the period 2017–2018 and the number (n) and percentage (%) resistant to the combination of cefalotin/cephazolin plus gentamicin.

Organism	Total	Resistant	
		(n)	(%)
Coagulase-negative staphylococci	173	12	7
Pseudomonas aeruginosa	50	0	0
Staphylococcus aureus[a]	43	3	7
Moraxella spp.[b]	24	0	0
Streptococcus pneumoniae	14	0	0
Corynebacterium spp.[b]	12	0	0
Micrococcus luteus	10	0	0
Serratia marcescens	9	0	0
Other gram-positive[c]	9	0	0
Streptococcus mitis/oralis group	8	2	25
Other gram-negative[d]	8	0	0
Bacillus spp.	6	0	0
Rothia spp.	3	0	0
Haemophilus influenzae[b]	3	1	33
Streptococcus gordonii	2	0	0
Total	**374**	**18**	**4.8**

a Including methicillin-resistant *Staphylococcus aureus*.

b *Moraxella* spp., *Corynebacterium* spp. and *Haemophilus influenzae* are not calibrated for gentamicin susceptibility testing by the CDS method. Isolates were therefore analysed as either sensitive or resistant on the basis of their susceptibility to cefalotin/cephazolin.

c Other gram-positive: *Abiotrophia defectiva* (1), *Clostridium sporogenes* (1), *Lysinibacillus sphaericus* (1), *Propionibacterium* spp. (1), *Enterococcus faecalis* (1), *Brevibacillus borstelensis* (1), *Paenibacillus* spp. (1), *Streptococcus sanguinis* (1), *Streptococcus dysgalactiae* (1).

d Other gram-negative: *Acinetobacter* spp. (1), *Achromobacter xylosoxidans* (1), *Enterobacter cloacae* complex (1), *Klebsiella oxytoca* (1), *Roseomonas mucosa* (2), *Citrobacter koseri* (1), *Proteus mirabilis* (1).

Table 9. The total number of organisms isolated from corneal scrapes during the period 2017–2018 and the number (n) and percentage (%) resistant to the combination of chloramphenicol plus gentamicin.

Organism	Total	Resistant	
		(n)	(%)
Coagulase-negative staphylococci	173	5	3
Pseudomonas aeruginosa	50	0	0
Staphylococcus aureus[a]	43	0	0
Moraxella spp.[b]	24	0	0
Streptococcus pneumoniae	14	1	7
Corynebacterium spp.[b]	12	0	0
Micrococcus luteus	10	0	0
Serratia marcescens	9	0	0
Other gram-positive[c]	9	1	11
Streptococcus mitis/oralis group	8	0	0
Other gram-negative[d]	8	0	0
Bacillus spp.	6	0	0
Rothia spp.	3	0	0
Haemophilus influenzae[b]	3	1	33
Streptococcus gordonii	2	0	0
Total	**374**	**7**	**2**

a Including methicillin-resistant *Staphylococcus aureus*.

b *Moraxella* spp., *Corynebacterium* spp. and *Haemophilus influenzae* are not calibrated for gentamicin susceptibility testing by the CDS method. Isolates were therefore analysed as either sensitive or resistant on the basis of their susceptibility to chloramphenicol.

c Other gram-positive: *Abiotrophia defectiva* (1), *Clostridium sporogenes* (1), *Lysinibacillus sphaericus* (1), *Propionibacterium* spp. (1), *Enterococcus faecalis* (1), *Brevibacillus borstelensis* (1), *Paenibacillus* spp. (1), *Streptococcus sanguinis* (1), *Streptococcus dysgalactiae* (1).

d Other gram-negative: *Acinetobacter* spp. (1), *Achromobacter xylosoxidans* (1), *Enterobacter cloacae* complex (1), *Klebsiella oxytoca* (1), *Roseomonas mucosa* (2), *Citrobacter koseri* (1), *Proteus mirabilis* (1).

Table 10. Combined resistance to chloramphenicol plus ciprofloxacin/ofloxacin: the total number of organisms isolated from corneal scrapes during the period 2017–2018 and the number (n) and percentage (%) resistant to the combination of chloramphenicol plus ciprofloxacin

Organism	Total	Resistant	
		(n)	(%)
Coagulase-negative staphylococci	173	3	2
Pseudomonas aeruginosa	50	0	0
Staphylococcus aureus[a]	43	0	0
Moraxella spp.	24	0	0
Streptococcus pneumoniae	14	0	0
Corynebacterium spp.	12	1	8
Micrococcus luteus	10	0	0
Serratia marcescens	9	1	11
Other gram-positive[b]	9	0	0
Streptococcus mitis/oralis group	8	0	0
Other gram-negative[c]	8	0	0
Bacillus spp.	6	0	0
Rothia spp.	3	0	0
Haemophilus influenzae	3	0	0
Streptococcus gordonii	2	0	0
Total	**374**	**5**	**1**

a Including methicillin-resistant *Staphylococcus aureus*.

b Other gram-positive: *Abiotrophia defectiva* (1), *Clostridium sporogenes* (1), *Lysinibacillus sphaericus* (1), *Propionibacterium* spp. (1), *Enterococcus faecalis* (1), *Brevibacillus borstelensis* (1), *Paenibacillus* spp. (1), *Streptococcus sanguinis* (1), *Streptococcus dysgalactiae* (1).

c Other gram-negative: *Acinetobacter* spp. (1), *Achromobacter xylosoxidans* (1), *Enterobacter cloacae* complex (1), *Klebsiella oxytoca* (1), *Roseomonas mucosa* (2), *Citrobacter koseri* (1), *Proteus mirabilis* (1).

Discussion

The use of combination therapy in the empirical management of BK is to offer the broadest spectrum of antibacterial cover, including both gram-positive and -negative organisms. To optimise clinical outcomes, empiric therapeutic regimens for infections should be informed, where possible, with local and quality assured AMR data. For the empiric treatment of clinical bacterial keratitis, there is a lack of recent and longitudinal AMR data to inform guidelines. In 2016, an international survey of corneal specialists sought to determine the clinical practice patterns for the empiric treatment of bacterial keratitis.[27] In 2016 an international survey of corneal specialists identified regional variations in practice influenced by drug availability, toxicity, broad spectrum coverage and resistance.[27]

In our study, 16% of *Staphylococcus aureus* isolates (7/43) were resistant to ciprofloxacin, higher than the rate reported in the Australian community in 2017 for blood isolates (6.2%), and for isolates from other sources (4.5%).[3] All *Streptococcus pneumoniae* isolates were susceptible to cefalotin and ciprofloxacin, indicating lower resistance than the background rates in isolates from other sites reported from the Australian community in 2017 (3.9% for blood culture isolates).[3] All *Pseudomonas aeruginosa* isolates were susceptible to ciprofloxacin and gentamicin. This differs from the AMR rates in the Australian community in 2017, where the AMR rate for ciprofloxacin was 6.4% and for gentamicin 5.7%. We note, however, that the numbers of isolates in our study were small relative to the national data set and from a single site, so differences might be expected.

In the United States, the Antibiotic Resistance Monitoring in Ocular micRoorganisms (ARMOR) surveillance studies report trends in antibiotic resistance of organisms isolated from ocular infections, providing data to assist health care practitioners in making informed choices regarding the treatment of ocular infections

with ophthalmic antibiotics. However, they do not provide recommendations for therapeutic combinations.[21]

A study from the United Kingdom, published in 2017, reported an analysis of AMR trends in isolates causing microbial keratitis at a tertiary hospital. In this study, similar empiric alternatives to our Centre were investigated, where ofloxacin was the first-line empiric therapy, and the second line cefuroxime plus gentamicin. The key findings included that ofloxacin remained an effective first line therapy; nonetheless, dual therapy offered broader coverage for clinical isolates in that setting. However, alternative, combination empiric antibiotic therapies were not investigated. There were no definitive recommendations for treatment and there was no further analysis of alternate antibiotic regimens in that study.[19]

In 2014, a systemic review and meta-analysis using Cochrane methodology was undertaken to evaluate the efficacy of topical antibiotics in the management of bacterial keratitis. This review identified that, despite numerous clinical trials, there remained a lack of consensus as to which topical antibiotics, and which regimen (i.e., monotherapy or combination therapy) provided superior outcomes. Whilst there was no evidence of difference in comparative efficacy between fluoroquinolone monotherapy and aminoglycoside–cephalosporin combination treatment options in the management of BK, there were differences in safety profiles. When compared with aminoglycoside–cephalosporin combination therapy, fluoroquinolones had benefits in terms of reduced ocular discomfort and chemical conjunctivitis, but an increased risk of white corneal precipitates with the use of ciprofloxacin drops.[28]

These studies indicate that empiric recommendations for bacterial keratitis vary with regards to fluroquinolone monotherapy versus combination therapy, without clear superiority demonstrated in clinical studies. The prime recommendation for empiric topical antibiotic in Australia, according to Therapeutic Guidelines – Antibiotic is with 0.3% ciprofloxacin or 0.3% ofloxacin.[23] In this study we compared AMR profiles from clinical isolates from patients with presumed bacterial keratitis covered with fluoroquinolone monotherapy (0.3% ciprofloxacin or 0.3% ofloxacin) or fortified combination therapy (5% cephazolin plus 0.9% gentamicin; chloramphenicol 0.5% plus gentamicin 0.9%; or 0.5% chloramphenicol plus 0.3% ciprofloxacin or ofloxacin).

In the present study, as with the findings from elsewhere,[19,21] gram-positive bacteria (coagulase-negative staphylococci, *Staphylococcus aureus* and *Streptococcus pneumoniae*) were the most commonly-isolated organisms from the cornea and accounted for 75% of isolates. Our detection of *Pseudomonas aeruginosa* in 13% of the total of 374 bacteria isolated is important, as it provides evidence that empiric topical antibiotic therapy must include an effective antipseudomonal agent rather than narrower approaches that target only commonly-isolated gram-positive bacteria.

Our findings support the current recommendations in Australia, *Therapeutic Guidelines – Antibiotic, Version 16, 2019*,[23] for the empiric, topical antibiotic therapy of bacterial keratitis. In our setting, there was greater *in vitro* antibiotic coverage with combinations including chloramphenicol and an anti-pseudomonal agent (either gentamicin or a fluoroquinolone). The combination of chloramphenicol 0.5% plus gentamicin 0.9% was statistically better than the regimens of either 0.3% ciprofloxacin/0.3% ofloxacin ($p = 0.007$) or fortified 5% cephazolin plus 0.9% gentamicin ($p = 0.005$). The combination of 0.5% chloramphenicol plus 0.3% ciprofloxacin/0.3% ofloxacin was also superior to the regimens of 0.3% ciprofloxacin/0.3% ofloxacin monotherapy ($p \leq 0.001$) and 5% cephazolin plus 0.9% gentamicin ($p = 0.003$).

Whilst our data represents a limited sample size, it demonstrates an overall fluoroquinolone resistance of 5.3% (95% CI: 3.1–7.6%); rates for cefalotin/cefazolin plus gentamicin were similar, with an overall rates of resistance of 4.8% (95% CI: 2.6–7.0%) while the combinations of

chloramphenicol plus gentamicin resistance was 1.9% (95% CI: 0.5–3.2%), and chloramphenicol plus ciprofloxacin/ofloxacin was 1.3% (95% CI: 0.2–2.5%).

In 2020, the lack of monitoring of AMR in ophthalmic practice seems unwise in this era of otherwise well-placed caution. It is known that susceptibility patterns change according to climate and geographical region, and can fluctuate over time.[29,30] A coordinated national program is urgently needed across Australia to provide wider-scale information on AMR in bacterial keratitis.

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Conflict of interest

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