Trends in antibiotic resistance in bacterial keratitis isolates from South India

Prajna Lalitha, MD[1], **Geetha Manoharan**[1], **Rajaram Karpagam**[1], **Namperumalsamy V. Prajna, MD**[2], **Muthiah Srinivasan, MD**[2], **Jeena Mascarenhas, MD**[2], **Manoranjan Das, MD**[2], **Travis C. Porco, PhD, MPH**[3,4,5], **Thomas M. Lietman, MD**[3,4,5], **Vicky Cevallos, MT**[3], **Jeremy D. Keenan, MD, MPH**[3,4]

[1]Department of Ocular Microbiology, Aravind Eye Care System, Madurai, India;

[2]Department of Cornea and External Diseases, Aravind Eye Care System, Madurai, India;

[3]Francis I. Proctor Foundation, University of California, San Francisco, USA

[4]Department of Ophthalmology, University of California, San Francisco, USA

[5]Department of Epidemiology & Biostatistics, University of California, San Francisco, USA

Abstract

Aims: To report trends in antibiotic resistance in cases of bacterial keratitis from a large eye hospital in South India.

Methods: In this retrospective cross-sectional study, the microbiology laboratory records of patients with infectious keratitis diagnosed at an eye hospital in South India from 2002–2013 were reviewed to determine the proportion with antibiotic non-susceptibility.

Results: 3685 bacterial isolates had susceptibility testing performed over the 12-year period. The two most common organisms with resistance results were *Streptococcus pneumoniae* (N=1204) and *Pseudomonas* aeruginosa (N=894). Antibiotic non-susceptibility was generally uncommon for these two organisms, and no significant trends were detected over the course of the study. In contrast, *Staphylococcus aureus* (N=211) isolates demonstrated a significant increase in fluoroquinolone non-susceptibility over the 12-year study period. This coincided with a significant increase in methicillin-resistant *S. aureus* (MRSA) during the study period, though the increase in fluoroquinolone resistance was likewise seen in methicillin-sensitive *S. aureus* (MSSA). For example, ofloxacin resistance in MSSA increased from 11.1% in 2002 to 66.7% in 2013 (P=0.002). No trends were apparent for the aminoglycocides, cefazolin, or vancomycin, for which *in vitro* non-susceptibility generally appeared to be low.

Conclusions: Resistance to antibiotics was generally stable for infectious keratitis isolates from a large eye hospital in South India, except for *S. aureus,* which experienced a significant increase in fluoroquinolone resistance from 2002 to 2013. Fluoroquinolone antibiotics currently have poor

Corresponding Author: Jeremy Keenan, 513 Parnassus Avenue, Med Sci S334B, San Francisco, CA 94143, jeremy.keenan@ucsf.edu, Tel: +1-415-476-6323, Fax: +1-415-476-0527.

in vitro activity against both methicillin-resistant and methicillin-sensitive *S. aureus* in South India and are therefore not the ideal therapy for Staphylococcal corneal ulcers.

INTRODUCTION

Corneal ulceration is a leading cause of blindness worldwide. The incidence of corneal ulceration is estimated to be between 113 and 799 per 100,000 person years in south Asia—at least ten-fold higher than in the United States.[1–3] Treatment for corneal infections is based on appropriate antimicrobial therapy, which requires knowledge of the local antimicrobial susceptibility patterns of various antibiotics. There are reasons to think that this susceptibility profile may have changed over recent years. For example, oral antibiotic consumption has increased in India recently, and especially the use of cephalosporins has increased.[4 5] This increased antibiotic consumption may have selected for resistant strains of bacteria, and therefore changed the susceptibility profile. Despite the burden of corneal ulcers in India, recent surveillance data from the Indian subcontinent is lacking. In this study, we investigate for trends in antimicrobial resistance in corneal ulcers seen at a large tertiary care eye hospital in South India.

METHODS

We reviewed the records of the Microbiology Laboratory at the Aravind Eye Hospital Madurai to identify all bacterial cultures performed for corneal ulceration from 2002 to 2013. Aravind Eye Hospital Madurai is the largest eye hospital in the state of Tamil Nadu in South India, and serves both a primary care and referral population. Corneal ulcers were routinely scraped and plated on blood, chocolate, and potato dextrose agar. Blood and chocolate plates were incubated at 37°C and examined for growth daily for 7 days; potato dextrose plates were incubated at 27°C and examined daily for 3 weeks. Anaerobic cultures were not routinely performed. Bacterial isolates from corneal scrapings were tested for susceptibility to a panel of antibiotics with a disc diffusion assay (Himedia, India) according to the manufacturer's recommendations, using resistance breakpoints according to guidelines from the Clinical and Laboratory Standards Institute (CLSI) or from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) if CLSI guidelines were not available.[6] CLSI and EUCAST recommend an MIC assay for vancomycin resistance in *Staphylococci*, but this was not performed due to limited resources. Instead, a disc diffusion assay was performed for vancomycin resistance in *Staphylococci*, using the breakpoints from viridans group *Streptococci*. CLSI currently recommends inferring beta-lactam susceptibility from the results of oxacillin susceptibility testing. However, because cefazolin is a major therapy used for corneal ulcers, a disc diffusion assay for cefazolin was performed for Gram-positive isolates, using the CLSI breakpoint from *Enterobacteriacea* for all tested organisms. No CLSI guidelines exist for gatifloxacin resistance in non-urinary *Pseudomonas aeruginosa*, so breakpoints for *Enterobacteriacea* were used for determining gatifloxacin resistance in *P. aeruginosa*. The CLSI also does not provide resistance

breakpoints for the disc diffusion assay for *Nocardia* or diphtheroids, so for these organisms we used the same breakpoints that we used for *Staphylococci*.

We depicted the proportion of bacterial cultures non-susceptible (i.e., resistance or intermediate resistance) to each of the tested antibiotics as the moving average, incorporating the prior and subsequent year's numbers. We tested for differences in non-susceptibility between different antibiotics using an equality of proportions test. We tested for trends over time with Poisson regression, accounting for autocorrelation with time series bootstrap with a fixed width of two (*tsboot* package in R; 1000 repetitions; significance level of 0.05). Statistical analysis was done with the statistical software R 3.1.2. We obtained ethical approval for this study from the Aravind Institutional Review Board.

RESULTS

Of 17,948 patients with corneal ulcers who had cultures performed over the 12-year period, 3,615 (20.1%) had bacterial growth, 6218 (34.6%) had fungal growth, 261 (1.5%) had parasite growth, 113 (0.6%) had both bacterial and fungal growth, and the remainder (43.1%) had no growth on culture. Of 4,132 total bacterial isolates, 3,685 (89.2%) had susceptibility testing performed. The most commonly isolated bacteria with susceptibility results were *Streptococcus pneumoniae* (N=1204, 32.7%), *Pseudomonas aeruginosa* (N=894, 24.3%), *Nocardia species* (N=246, 6.7%), diphtheroids (N=235, 6.4%), viridans group *Streptococcus* (N=212, 5.8%), *Staphylococcus aureus* (N=197, 5.3%), coagulase negative *Staphylococci* (N=151, 4.1%), and *Enterobacteriaceae* (N=110, 3.0%); Table 1. The results of susceptibility testing are shown for each of the 12 years of the study in Supplementary Table 1 for Gram-positive organisms and Supplementary Table 2 for Gram-negative organisms; results for individual organisms are summarized below.

S. pneumoniae

Pneumococcal isolates from corneal ulcers generally remained susceptible to most antibiotics over the study period (Figure 1). Ofloxacin non-susceptibility was observed in 54 of 1201 (4.5%, 95%CI 3.4–5.8%) isolates over the entire study period; non-susceptibility was similar for other fluoroquinolones. Non-susceptibility to chloramphenicol was unusual (37 of 1204 isolates; 3.1%, 95%CI 2.2–4.2%) and vancomycin non-susceptibility was never observed (0%, 95%CI 0–0.3%). Thresholds for pneumococcal non-susceptibility to ciprofloxacin are not provided by the CLSI, but ciprofloxacin-resistant pneumococci were common when using the EUCAST criteria (290 of 1196 isolates; 24.2%, 95%CI 21.8–26.8%). Screening pneumococcal isolates for oxacillin resistance started in 2009; non-susceptibility was observed in 82 of 580 (14.1%, 95%CI 11.4 to 17.2%) isolates. Using the *Enterobacteriacea* breakpoint for cefazolin resistance, non-susceptibility was found in 5 of 1204 (0.4%, 95%CI 0.1 to 1.0%) isolates. Resistance to each of the tested antibiotics was stable over the 12-year duration of the study.

P. aeruginosa

Ofloxacin non-susceptibility was seen in 116 of 887 (13.1%, 95%CI 10.9 to 15.5%) *Pseudomonas* corneal ulcers throughout the study period (Figure 2), with similar rates of

non-susceptibility among the other tested fluoroquinolones. Non-susceptibility was similar for the aminoglycocides; for example, 121 of 894 (13.5%, 95%CI 11.4 to 16.0%) isolates were resistant to gentamicin over the study period. Non-susceptibility to ceftazidime was less common (36 of 651 isolates resistant; 5.5%, 95%CI 3.9 to 7.6%); *P*<0.001 compared with ofloxacin or gentamicin non-susceptibility. Resistance patterns were relatively stable over the duration of the study for all tested antibiotics.

Nocardia species

Resistance in Nocardia species was estimated from the disc diffusion assay using CLSI guidelines for *Staphylococci*. As shown in Figure 3, moderate levels of non-susceptibility were observed for all tested antibiotics except for amikacin (0 of 246 isolates classified as resistant; 95%CI 0–1.5%). Using the *Enterobacteriacea* breakpoint, 209 of 241 (86.7%, 95%CI 81.8 to 90.7%) *Nocardia* isolates were non-susceptible to cefazolin. Using the viridans group *Streptococci* breakpoint, 134 of 201 (66.7%, 95%CI 59.7 to 73.1%) were non-susceptible to vancomycin. No trends in antibiotic non-susceptibility were apparent for the study duration.

Diphtheroids—Resistance thresholds for diphtheroids are not available from CLSI, so we used the CLSI criteria for *Staphylococci*. Using these criteria, non-susceptibility to ofloxacin was seen in 46 of 221 (20.8%, 95%CI 15.7 to 26.8%) isolates, which was significantly less than each of the other fluoroquinolones except for gatifloxacin (*P*<0.001 when compared to ciprofloxacin, moxifloxacin, or levofloxacin; P=0.54 when compared to gatifloxacin). Using the *Enterobacteriacea* breakpoint for cefazolin resistance and viridans group *Streptococci* breakpoint for vancomycin resistance, non-susceptibility was found in 51 of 221 (23.1%, 17.7 to 29.2%) and 5 of 221 (2.3%, 95%CI 0.7 to 5.2%) diphtheroid isolates, respectively. Resistance did not markedly change over the study duration for any of the tested antibiotics (Figure 3).

Viridans group *Streptococci*—Fluoroquinolone non-susceptibility was more common for viridans group *Streptococci* than it was for *S. pneumonia*, with ofloxacin non-susceptibility observed in 46 of 212 (21.7%, 95%CI 16.3 to 27.9%) isolates (Figure 1; *P*<0.001 compared with proportion of *S. pneumoniae* non-susceptible to ofloxacin). Chloramphenicol non-susceptibility was uncommon and vancomycin non-susceptibility was never observed. Using the *Enterobacteriacea* breakpoint for cefazolin resistance, non-susceptibility was found in 12 of 212 (5.7%, 95%CI 3.0 to 9.7%) isolates.

Methicillin resistant *S. aureus* (MRSA)—We identified MRSA using the oxacillin disk diffusion assay starting in 2005. MRSA was not present from 2005 until 2007, but accounted for 1 of 22 (4.5%) *S. aureus* isolates in 2008, 4 of 27 (14.8%) isolates in 2009, 2 of 20 (10.0%) isolates in 2010, 5 of 24 (20.8%) isolates in 2011, 5 of 27 (18.5%) isolates in 2012, and 3 of 15 (20.0%) in 2013 (*P*=0.002 testing for a trend over time). Most of the MRSA isolated during the study period was also resistant to the fluoroquinolone and aminoglycoside antibiotics (Figure 4). Only a single isolate was resistant to chloramphenicol. No isolates were non-susceptible to vancomycin when classified using the breakpoints for viridans group *Streptococci*.

Methicillin-susceptible *S. aureus* (MSSA)—Fluoroquinolone non-susceptibility was common in *S. aureus*, even when only the methicillin-susceptible isolates were analyzed. For example, ofloxacin non-susceptibility was observed in 75 of 177 (42.4%, 95%CI 35.0–50.0%) MSSA isolates. Moreover, the proportion of methicillin-susceptible isolates resistant to most fluoroquinolones increased over the duration of the study (*P*=0.03 for ciprofloxacin, *P*=0.002 for ofloxacin, *P*=0.007 for moxifloxacin, *P*=0.20 for gatifloxacin, and *P*=0.42 for levofloxacin; Figure 1). By 2013, fluoroquinolone non-susceptibility was present in 26–66% of methicillin-susceptible *S. aureus* isolates. The aminoglycocides had variable activity against MSSA over the course of the study, with 43 of 177 (24.3%, 95%CI 18.2–31.3%) isolates resistant to gentamicin and similar numbers resistant to tobramycin. Using breakpoints for viridans group *Streptococci,* non-susceptibility to vancomycin was not observed in MSSA at any point during the study surveillance period.

Coagulase negative *Staphylococci*—Antibiotic resistance in coagulase negative *Staphylococci* was generally similar to *S. aureus*, with high levels of fluoroquinolone and aminoglycocide non-susceptibility (Figure 1). For example, 70 of 150 (46.7%, 95%CI 38.5–55.0%) isolates were resistant to ofloxacin, and 78 of 151 (51.7%, 95%CI 43.4–59.9%) were resistant to gentamicin. Testing for oxacillin resistance began in 2010; of 66 coagulase negative *Staphylococcal* isolates tested over the last 4 years of the study, 35 (53.0%, 95%CI 40.3–65.4%) were oxacillin-resistant. Unlike MSSA, no trends in fluoroquinolone non-susceptibility were observed over the duration of the study.

Enterobacteriaceae

Resistance among enteric Gram-negative rods was overall similar to that among *P. aeruginosa* (Figure 2). For example, non-susceptibility to ofloxacin was documented in 16 of 110 (14.5%, 95%CI 8.5–22.5%) *Enterobacteriaceae* isolates, and results for the other fluoroquinolones was similar. Gentamicin non-susceptibility was found in 21 of 110 isolates (19.1%, 95%CI 12.2–27.7%). Resistance was similar in other antibiotics with Gram-negative coverage, although amikacin non-susceptibility was slightly less common (12 of 110 isolates resistant; 10.9%, 95%CI 5.8–18.3%; *P*=0.03 compared with gentamicin non-susceptibility). The proportion of Enterobacteriaceae isolates resistant to the fluoroquinolones appeared to increase slightly over the duration of the study, although this was not statistically significant (e.g., *P* for trend over time=0.07 for ofloxacin).

DISCUSSION

In South India, the most common causes of bacterial keratitis generally remained susceptible to the fluoroquinolone antibiotics during the 2000s. The exceptions were *Nocardia species* and *Staphylococcus aureus*, which demonstrated considerable levels of non-susceptibility to each of the fluoroquinolones. The Gram-positive organisms other than *Nocardia* that were tested in this study generally remained susceptible to chloramphenicol, and universally remained susceptible to vancomycin, and the Gram-negative organisms generally remained susceptible to the aminoglycosides and ceftazidime. MRSA was seen more commonly over the 12 years of the study, as was fluoroquinolone non-susceptibility among methicillin-

susceptible *S. aureus.* Non-susceptibility to other organisms remained relatively stable over the duration of the study.

Methicillin and fluoroquinolone non-susceptibility each became more common in *S. aureus* over the duration of this study. These results are consistent with previous publications that have noted an increase in MRSA and an increase in fluoroquinolone resistance among ocular *S. aureus* species over the 1990s and 2000s.[7–13] These studies reported increases in staphylococcal ciprofloxacin and/or ofloxacin resistance during the 1990s, with up to 40% of isolates demonstrating fluoroquinolone resistance by the early 2000s. Studies from South India have reported similarly high rates of ciprofloxacin resistance in *S. aureus* during the same time period (resistance in approximately 30% of isolates).[14 15] Previous studies from the United States in the early and mid 2000s have demonstrated fluoroquinolone resistance in approximately 80% of MRSA isolates, but in less than 20% of MSSA isolates. [9 16 17] In contrast, we found a significant increase in fluoroquinolone non-susceptibility even among MSSA in this South Indian study, with 60–66% of isolates resistant to ciprofloxacin, ofloxacin, or moxifloxacin, and 26–46% of isolates resistant to gatifloxacin or levofloxacin in 2013. Aminoglycoside non-susceptibility was also much higher in the MSSA isolates from this South Indian population than has been reported previously in the United States, suggesting variability in the clonal structure of *S. aureus* populations in different locations.[9 16] The statistically significant increase in fluoroquinolone non-susceptibility among MSSA isolates over the 12 years of the study may be due to increased use of topical fluoroquinolone antibiotics in India, although this theory is speculative. Of note, *S. aureus* generally remained susceptible to chloramphenicol, suggesting that this drug may be an appropriate choice in South India—especially if vancomycin is not available.

S. pneumoniae isolates displayed more in vitro non-susceptibility to ciprofloxacin than to the other fluoroquinolones. This finding is consistent with some surveillance studies, [18 19] although not others.[16 20] In vitro studies suggest that ciprofloxacin may be more likely to select for resistant pneumococci compared to other fluoroquinolones.[21 22] Compared with the other fluoroquinolones, ciprofloxacin has a lower binding affinity for its enzymatic targets in *S. pneumonia* (DNA topoisomerase IV and DNA gyrase),[23–25] is more likely to develop resistance through a single mutation,[26] and has a less bulky chemical structure that may allow for easier efflux.[16 27] In addition, ciprofloxacin has been used in India for longer than the other fluoroquinolones, so bacteria have likely been subject to greater selection pressure from ciprofloxacin than from the other fluoroquinolones. Regardless of the mechanism, the results of this study confirm that ciprofloxacin should not be used as the first-line therapy for pneumococcal corneal ulcers in South India, and would not be the best choice for empiric antibiotic therapy when microbiologic testing is inconclusive or not available.

When resistance breakpoints for *Staphylococcus* were used for *Nocardia*, none of the *Nocardia* isolates were found to be resistant to amikacin. In contrast, *Nocardia* species exhibited high levels of non-susceptibility to the fluoroquinolones, cefazolin, gentamicin, and tobramycin. Amikacin was first reported as a treatment for *Nocardia* keratitis in 1996. [28] Subsequent studies of *Nocardia* keratitis isolates, some of which include isolates also described in the current report, have demonstrated near-universal susceptibility to amikacin.

[29–31] In the current study, *Nocardia* isolates remained susceptible to amikacin for the entire study period, further supporting amikacin as the first line treatment for *Nocardia* keratitis. It is important to note that the CLSI recommends broth microdilution to test Nocardia resistance, whereas in this study we used a disc diffusion assay and applied breakpoints from *Staphylococci*. However, our results are supported by other studies that have found little *in vitro* resistance to amikacin when using broth microdilution.[32 33] Because amikacin seems to have similar efficacy as the other aminoglycocides for non-*Nocardia* infections and superior efficacy for *Nocardia* corneal ulcers, and because of the relatively high proportion of *Nocardia* corneal ulcers in South India, amikacin would be a good choice for empiric aminoglycocide therapy in this location.

Although not currently recommended by the CLSI, we performed cefazolin susceptibility testing for Gram-positive organisms since this antibiotic is so commonly used for corneal ulcers. In general, cefazolin resistance was uncommon for most of the organisms included in this report, except for *Nocardia species* and diphtheroids. The relationship between disc diffusion results and clinical efficacy when an antibiotic is applied topically for corneal ulcers is not clear, especially since the concentration of the antibiotic when administered as a fortified topical antibiotic is so high. Although the cefazolin results in this report must be interpreted with caution, it is promising that so little non-susceptibility was observed for this antibiotic.

A major strength of this study is the large sample size, which allowed assessment of individual bacterial species with relatively high precision. Other strengths include the variety of antibiotics for which susceptibility was tested and the long duration of the study. Moreover, we accounted for autocorrelation between adjacent time points when assessing for temporal trends, which provides a more accurate *P*-value and has rarely been done in previous studies. There are also several limitations. Most importantly, this is a single-center study, and therefore the resistance patterns cannot necessarily be extrapolated outside of South India. Regardless, the results have important implications given the large population of South India and the high rate of corneal ulcers there. The study is a retrospective analysis of antibiotic susceptibility tests that were done as part of routine clinical practice, and is therefore subject to the biases inherent in such studies. The study includes only *in vitro* resistance, and like all such studies, defines susceptibility breakpoints according to published standards. These standards are based on the correlation between systemic antibiotic concentrations and clinical response for non-ocular disease, and may be different for topically applied antibiotics, which typically can attain much higher concentrations than systemic administration.[34] Finally, Aravind Eye Hospital is a referral center, so the susceptibility patterns of this study may be different from that of the general population in South India.

In conclusion, this study from South India found that fluoroquinolone antibiotics other than ciprofloxacin generally would offer similar empiric coverage for Gram-positive and Gram-negative organisms as would the aminoglycocides and cefazolin. The proportion of isolates identified as MRSA increased over the latter years of the study but nonetheless remained a minority of the *S. aureus* that was cultured. Fluoroquinolone non-susceptibility in MSSA increased over the course of the study but the susceptibility of MSSA to chloramphenicol

and vancomycin remained high. Culture and susceptibility testing are important in south India, where roughly 15–20% are not susceptible to typically used empiric therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding

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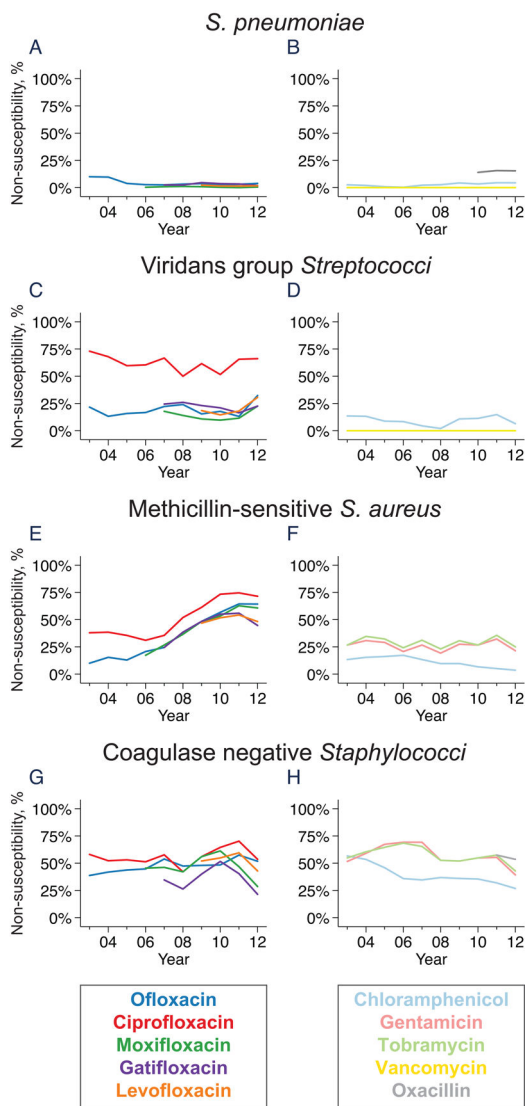
Figure 1. Trends in antibiotic resistance among Gram-positive organisms.
The graphs represent the moving average of the annual proportion of non-susceptible isolates for *S. pneumoniae* (parts A and B), viridans group *Streptococci* (parts C and D), methicillin-susceptible S. aureus (parts E and F), and coagulase negative Staphylococci (parts G and H).
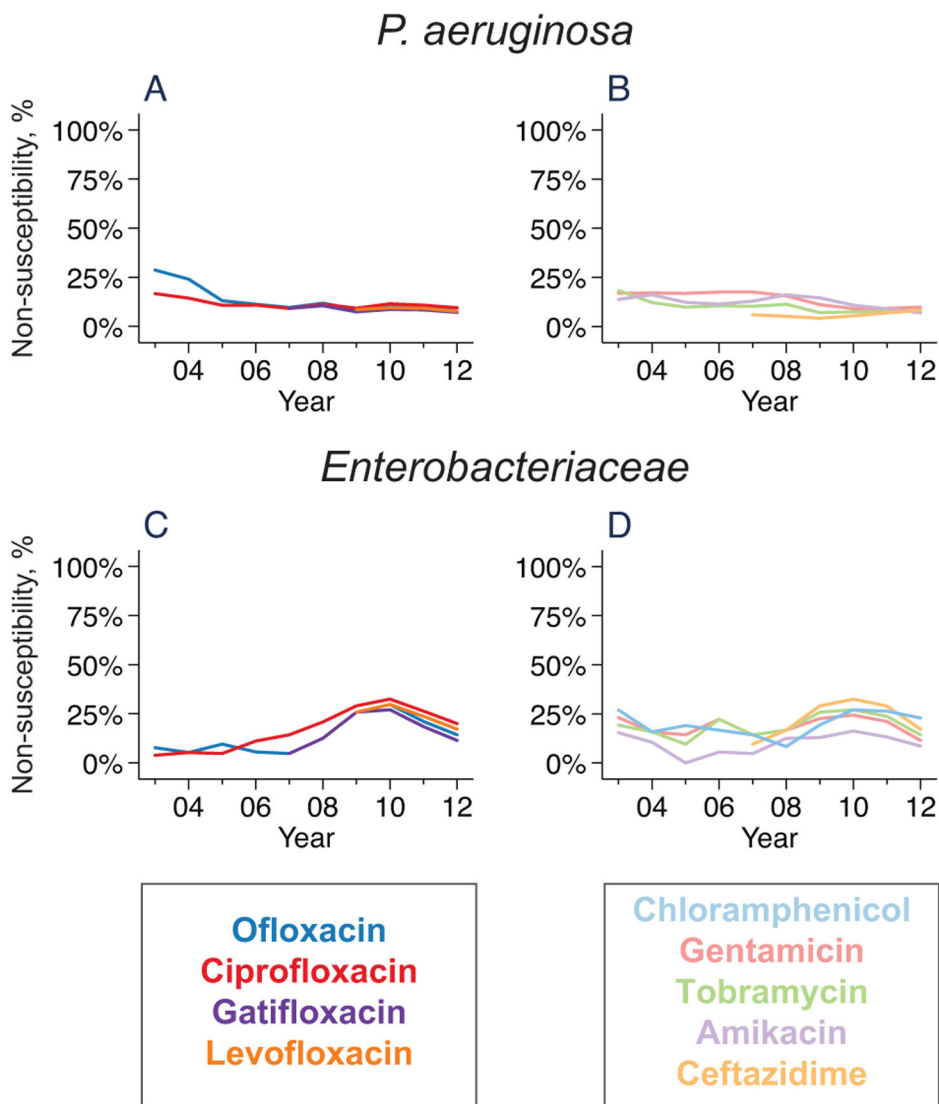
Figure 2. Trends in antibiotic resistance among Gram-negative organisms.
Lines represent the moving average of the proportion of non-susceptible isolates for *P. aeruginosa* (parts A and B) and *Enterobacteriaceae* (parts C and D).

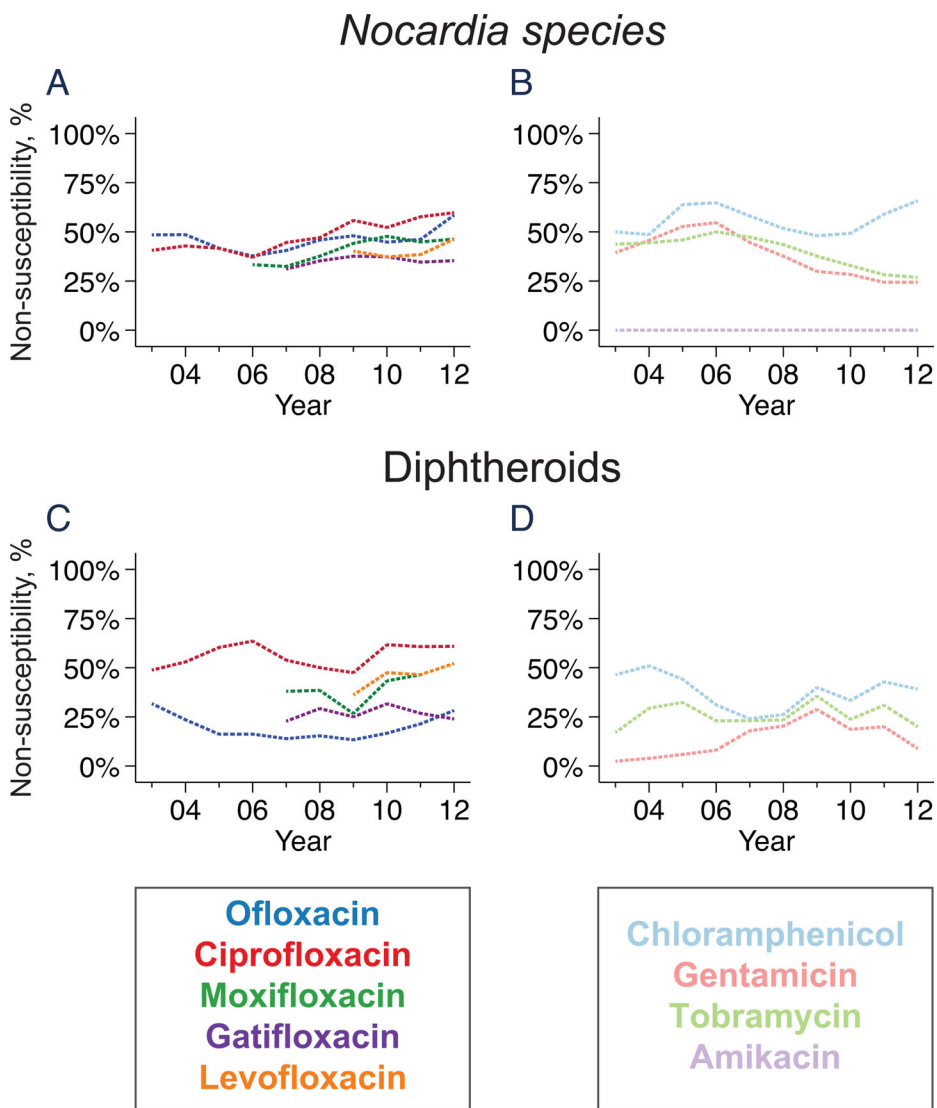## Nocardia species

Diphtheroids

Figure 3. Trends in antibiotic resistance among *Nocardia* (parts A and B) and diphtheroids (parts C and D).

Lines represent the moving average of the proportion of isolates resistant to each antibiotic; lines are dashed to indicate that established breakpoints for the disc diffusion assay do not exist for either organism.

No.	CIP	OFL	GAT	LEV	MXF	GEN	TOB	CHL
13	R	R	I/R*	I/R*	R	R	R	S
3	R	R	I/R**	R	R	S	S	S
1	R	R	R	R	R	R	R	R
1	R	S	R	I	R	R	R	S
1	R	R	S	R	R	R	R	S
1	R	R	I	S	I	R	R	S

Figure 4. Spectrum of resistance in the 20 methicillin resistant *Staphylococcus aureus* isolates identified at Aravind Eye Hospital, 2007–2013.

CHL = chloramphenicol; CIP = ciprofloxacin; GAT = gatifloxacin; GEN = gentamicin; I = intermediate susceptibility; LEV = levofloxacin; MOX = moxifloxacin; OFL = ofloxacin; R = resistant; S = susceptible; TOB = tobramycin

*Two isolates had intermediate susceptibility to levofloxacin, 3 isolates had intermediate susceptibility to gatifloxacin, and 2 isolates had intermediate susceptibility to both levofloxacin and gatifloxacin

**One isolate had intermediate susceptibility to gatifloxacin

Table 1.

Number of organisms with susceptibility test results each year over the 12-year study period.

Testing for methicillin-resistant *S. aureus* started in 2005.

Organism	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Gram-positive												
S. pneumoniae	55	52	86	59	89	148	121	107	124	97	137	129
Viridans group *Streptococci*	5	14	18	21	18	9	18	23	24	15	22	25
Methicillin-resistant *S. aureus*	---	---	---	0	0	0	1	4	2	5	5	3
Methicillin-sensitive *S. aureus*	9	11	10	5	16	8	21	23	18	19	22	15
Coagulase negative *Staphylococci*	7	10	14	20	15	4	7	8	10	13	24	19
Nocardia species	9	12	12	12	13	29	32	24	21	22	35	25
Diphtheroids	8	17	16	18	34	22	23	20	17	23	16	21
Gram-negative												
P. aeruginosa	52	50	58	74	82	84	107	66	97	80	73	71
Enterobacteriaceae	10	7	9	3	9	6	6	12	13	12	13	10