Bacterial Susceptibility to Topical Antimicrobials and Clinical Outcome in Bacterial Keratitis

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PURPOSE. To investigate the relationship between the susceptibility of bacteria to topical antimicrobials and clinical outcome in microbial keratitis.

METHODS. Clinical outcome data were collected from patients with microbial keratitis from whom a bacterium had been isolated during the period 2003 to 2006. The minimum inhibitory concentration (MIC) was determined for the isolates against 10 antimicrobials. The determinants of the primary clinical outcome, the ratio of healing time (closure of epithelial defect) to ulcer size (HT/UA), was analyzed in a general linear model.

RESULTS. Complete clinical outcome and MIC data were available for 421 patients. Sixteen (4%) patients required enucleation and 23 (5%) surgical treatment; in 382 (91%) the ulcer healed with intensive topical antimicrobial therapy. There were significant correlations between HT/UA and organism type ($P = 0.001$), nearest distance of the ulcer to the limbus (0.02), and MIC of the first antimicrobial used or lowest MIC of combined therapy ($P = 0.006$). In a model including patients who received monotherapy with a fluoroquinolone who had no subsequent change in their treatment and whose ulcers healed without surgical intervention, there were significant linear associations between clinical outcome and MIC for *Pseudomonas* spp. ($P = 0.047$), *Staphylococcus aureus* ($P = 0.04$), and *Enterobacteriaceae* ($P = 0.045$), but not for *Streptococcus* spp. ($P = 0.85$) and coagulase-negative staphylococci (CNS) ($P = 0.88$).

CONCLUSION. With fluoroquinolone monotherapy, there was significant association between the MIC of the antimicrobial prescribed and the clinical outcome with all bacteria except CNS and *Streptococcus* spp. The approach used in this study, if used prospectively, could allow topical breakpoint susceptibility concentrations to be determined for individual antimicrobial and bacterial combinations. (*Invest Ophthalmol Vis Sci.* 2010;51:362–368) DOI:10.1167/iovs.09-3933

Microbial keratitis is a major cause of corneal opacity and loss of vision worldwide.[1–6] The most common causative organisms are bacteria, although fungi and protists are also pathogens.[7–11] The spectrum of causative organisms and their susceptibility to antimicrobials varies according to the latitude and the degree of urbanization of the population studied.[7,9–13] Several specific risk factors for infection have been identified.[2,7–9,11,13–15] In developed countries in temperate latitudes, bacteria are the most common isolates.[12] The normal management strategy if microbial infection is suspected is to collect samples of corneal tissue for culture and then to initiate antimicrobial treatment empirically.[11,16–22] The choice of treatment is based on the use of a single antimicrobial, or a combination of antimicrobials, that provides a broad range of activity against both Gram-positive and -negative bacteria. Once an isolate has been obtained, it is then possible to modify the treatment according to the in vitro susceptibility pattern of the isolate.

The clinical outcome in microbial keratitis is likely to be dependent on the virulence of the infecting bacteria, the minimum inhibitory concentration (MIC) of the antimicrobial against the bacteria, as well as host factors such as the presence of ocular surface disease or the host's immune system. The relative contribution of the MIC in determining the response to therapy is not clear. Wilhelmus et al.[11] reported that patients whose principal corneal isolate had a ciprofloxacin MIC exceeding 1 mg/L improved significantly more slowly than those with a more susceptible isolate, and, more recently, Chen et al.[23] reported a study with 34 patients in which a significant relationship was found between MIC and scar size but not healing time. The MIC and disc susceptibility criteria used to choose the antimicrobial for treatment are based on the anticipated response of the bacteria against concentrations of the antimicrobial that can be achieved in serum.[24–26] Topical application of an antimicrobial to the cornea may achieve a very different concentration and bioavailability in the tissue than the serum levels.[27] There is limited information about the in vitro response of common isolates from cases of bacterial keratitis to concentrations of antimicrobial that can be expected with intensive topical use, and thus the appropriate disc susceptibility breakpoint for each antimicrobial and bacterial isolate combination has not been determined.

We have collected a panel of bacterial isolates from cases of microbial keratitis from ophthalmology specialist units within the United Kingdom. The MICs of the isolated bacteria have been determined against a panel of antimicrobials commonly available for topical ophthalmic use. The MIC results have been analyzed in combination with retrospectively collected clinical

TABLE 1. Available Microbiological and Clinical Outcome Data

	Bacteria Isolated in Each Group *n* (%)	Bacteria Isolated in Each Group with MIC Data *n* (%)	Bacteria with Completed Clinical And MIC Data *n* (%)	Percentage of Each Bacterial Group with Completed Clinical and MIC Data
S. aureus	96 (13)	93 (13)	56 (13)	60
CNS	209 (27)	179 (25)	118 (28)	66
S. pneumoniae	26 (3)	26 (4)	12 (3)	46
Other *Streptococcus* spp.	77 (10)	75 (11)	47 (11)	63
Pseudomonas spp.	161 (21)	158 (22)	91 (22)	58
Enterobacteriaceae	108 (14)	101 (14)	56 (13)	55
Others*	87 (11)	78 (11)	41 (10)	53
Total	764 (100)	710 (100)	421 (100)	59

* *Acinetobacter* sp., *Stenotrophomonas* sp., *Haemophilus* spp., *Moraxella* spp., *Neisseria* spp., *Bacillus* sp., *Aeromonas* sp., *Corynebacterium*.

data to determine the relative contribution of the MIC value to the overall response to treatment.

METHODS

Bacteriology

Six specialist ophthalmology centers (Birmingham, Bristol, Liverpool, London, Manchester, and Newcastle) participated in the study. Bacterial isolates from patients who presented with suppurative keratitis were collected at each center during the period April 2003 to April 2006. Corneal scrape samples were placed onto agar culture plates and also into enrichment culture broth (e.g., brain heart infusion). The plates were incubated overnight at 37°C under both aerobic and enriched carbon dioxide (5%) atmospheric conditions in the local laboratory as previously described.[8,16] At each center, bacterial isolates were identified according to standard microbiologic methods: Gram's stain, colony morphology, and biochemical reactions. Disc susceptibility profile was determined according to the British Society of Antimicrobial Chemotherapy guidelines, which are the accepted standard methods for bacterial susceptibility testing in the United Kingdom.[26] Bacterial isolates from the six centers were then sent to one microbiology laboratory (Royal Liverpool University Hospital NHS Trust) where they were subcultured and stored on sterile beads (Protect Beads; TSC Ltd., Heywood, Lancashire, UK) at −80°C. Polymicrobial infections were not included, as in most instances it would not be possible to determine the causative bacterial species. This would then have made it difficult to determine the relationship between clinical outcome and the MIC of antimicrobial used to treat the causative bacterium.

At a later date, bacteria were recovered from storage by inoculating a bead onto blood agar (Oxoid, Basingstoke UK) and incubating over-

TABLE 2. Therapeutic Profiles Applied in Patients with Bacterial Keratitis

	FL %	CHL %	GM %	PG %	FC %	XM %	TP %	TZ %
Initial therapy								
Monotherapy	84	8	3	2	0	3	0	0
Combination	9	22	12	14	1	25	13	4
Changed therapy								
Monotherapy	27	38	16	0	0	13	1	5
Combination	15	20	30	0	0	25	10	0

FL, fluoroquinolones, including ciprofloxacin 0.3% and ofloxacin 0.3%; CHL, chloramphenicol; GM, 0.5% and 1%, gentamicin; PG, 0.3%, penicillin; FC, fucidic acid; 1%; XM, cefuroxime 5%; TP, teicoplanin 1%; TZ, ceftazidime.

night at 37°C. The MIC was determined using antimicrobial test strips (E-test; AB bioMérieux, Solna, Sweden), according to the manufacturer's instructions. In brief, a suspension of the organism was prepared in sterile water to a density equivalent to a 0.5 McFarland standard. The suspension was then evenly applied with a sterile swab to the surface of a 140-mm diameter plate containing agar (Iso-sensitest; Oxoid), with or without blood. The test strips for each antimicrobial were placed on inoculated agar plates (maximum, five per plate) and the plates incubated for 16 to 18 hours at 37°C under aerobic conditions.[28,29] The MIC for each antimicrobial was then recorded as the intersection of the zone of inhibition with the scale on the test strip. The 10 antimicrobials chosen were penicillin, cefuroxime, ceftazidime, chloramphenicol, amikacin, gentamicin, teicoplanin, vancomycin, ofloxacin, and ciprofloxacin. These antimicrobials included those prescribed and were selected, because a questionnaire sent to ophthalmologists in the United Kingdom suggested they were the most frequently prescribed for cases of microbial keratitis. The project was approved by the Moorfields Eye Hospital Research Ethics Committee and adhered to the tenets of the Declaration of Helsinki.

Clinical Data

The treatment protocol for suspected microbial keratitis at all centers was intensive topical application of an antimicrobial, although the choice of antimicrobial used varied among the centers. At each center, a standardized form was used to collect clinical data retrospectively from the clinical records. Complete data, including follow-up information, were only available for a subset of the patients. Data included ocular and systemic risk factors for infection, the antimicrobial used at the time of presentation, the first-line treatment prescribed, and changes in treatment and ancillary treatment such as the use of topical steroids or bandage contact lenses. To determine outcome, the following data were collected at the date of presentation and again at the date of healing: the size (orthogonal diameters of the major and minor axes) of the corneal ulcer[15] and scar, minimum distance from the limbus, duration of treatment, corneal surgery (e.g., application of corneal glue and tectonic or penetrating corneal graft), and loss of the eye (enucleation or evisceration). Healing time was defined as the interval until closure of the epithelial defect and treatment time as the interval during which antimicrobials were prescribed.

Data Analysis

Bacterial isolates were categorized into seven groups: *Staphylococcus aureus*, coagulase-negative staphylococci (CNS), *Pseudomonas* spp., *Enterobacteriaceae*, *Streptococcus pneumoniae*, other *Streptococcus* spp., and miscellaneous other bacteria. The categories were based on bacteria with similar microbiologic characteristics and likely susceptibility patterns. The proportions of each bacterial group were compared among the patients for whom full clinical data were available and

TABLE 3. Clinical Parameters of Those Patients Whose Ulcers Healed When Treated with the Main Groups of Bacteria

Bacterial Group	UA (mm²)	Limbal Distance (mm)	Scar Area (mm²)	Scar/Ulcer Ratio	TT (d)	HT (d)	HT/UA (d/mm²)
Pseudomonas spp.	9.58 (15.74)	2.65 (1.20)	7.20 (15.17)	0.72 (0.52)	23.62 (15.18)	15.23 (16.82)	3.75 (3.43)
CNS	4.62 (9.23)	2.54 (1.40)	2.74 (7.20)	0.70 (1.56)	17.33 (13.26)	11.52 (14.54)	6.30 (7.39)
S. aureus	5.89 (9.34)	2.54 (1.45)	3.80 (7.76)	0.77 (1.13)	21.31 (14.58)	14.57 (12.51)	5.34 (5.06)
Streptococcus spp.	6.52 (11.35)	2.56 (1.32)	4.65 (10.07)	0.86 (1.41)	23.23 (20.72)	16.02 (17.04)	5.56 (5.63)
Enterobacteriaceae	9.49 (19.15)	2.86 (1.49)	5.73 (14.98)	0.52 (0.64)	22.92 (18.25)	15.00 (18.35)	6.98 (10.22)

Mean (SD) treatment time (TT) and healing time (HT) in days. Major and minor axis lengths of ulcer and scar and nearest distance to the limbus in millimeters. HT/UA, ratio of healing time to ulcer area. Data for *S. pneumoniae* are not shown, as only 8 of the 12 healed, and too small a sample size skewed the data.

those for whom data were missing. The MIC data for the 10 antimicrobials was summarized for the principle groups of bacteria. For reference, published breakpoint concentrations derived for systemic infections were used, that is, the MIC below or above which organisms are categorized as susceptible or resistant, respectively.[26]

The eyes were grouped as either healed without surgical intervention, healed after surgical intervention, or requiring enucleation or evisceration (failure to heal). Most of the patients were initially treated with a fluoroquinolone (ciprofloxacin or ofloxacin) as monotherapy. In this subgroup of patients, the determinants of successful treatment (i.e., excluding patients who required surgical intervention) were explored using the clinical and susceptibility data in a general linear model. Because ulcer size was an important determinant of healing, the primary clinical outcome measure used was the ratio of the healing time to ulcer area (HT/UA). The logarithm of the MIC of the fluoroquinolone used was determined for each bacterial isolate and included in the general linear model. For those cases in which the MIC exceeded the highest measurable value, the next doubling in dilution was used for the analysis. The model was then tested separately for each of the principle groups of bacteria. Normally distributed data were summarized by the mean and SD. Fisher's exact test was used to compare proportions (all analyses by SPSS ver. 15; SPSS Inc, Chicago, IL).

RESULTS

Bacterial Isolates and Susceptibility Data

Bacteria were isolated from 764 cases of microbial keratitis (Table 1). CNS, *Streptococcus* spp., and *Pseudomonas* spp. were the commonest isolates. MIC data (range, MIC_{50} and MIC_{90} for each organism/antimicrobial combination) were available for 710 (93%) of the isolates (54 isolates could not be recovered from storage). The MIC_{90} of the *Pseudomonas* spp. and the *Enterobacteriaceae* for ceftazidime (2 and 1 mg/L, respectively), gentamicin (2 and 1 mg/L), amikacin (4 and 2 mg/L), ciprofloxacin (0.5 and 0.19 mg/L), and ofloxacin (1.5

and 0.5 mg/L) were lower or equal to their respective systemic breakpoint concentrations. For *S. pneumoniae*, the MIC_{90} for penicillin (0.02 mg/L), cefuroxime (0.03 mg/L), chloramphenicol (2 mg/L), vancomycin (1 mg/L), and teicoplanin (0.08 mg/L), were lower than and for ciprofloxacin (1.75 mg/L) and ofloxacin (4 mg/L) were higher than their respective systemic breakpoint concentrations. For the *S. aureus* the MIC_{90} for penicillin (1.5 mg/L) was below, and for chloramphenicol (8 mg/L), gentamicin (1 mg/L), amikacin (4 mg/L), ciprofloxacin (1.4 mg/L), and ofloxacin (0.95 mg/L) were close to or equal to their respective systemic breakpoints. Among the CNS the MIC_{90} for penicillin (1 mg/L), chloramphenicol (4 mg/L), gentamicin (1 mg/L), amikacin (3 mg/L), vancomycin (3 mg/L), teicoplanin (4 mg/L), and ciprofloxacin (0.5 mg/L) was lower than or equal to their systemic breakpoints, whereas for ofloxacin (1.5 mg/L) it was higher.

Although comparison between the MIC of the antimicrobials is limited by their differences in actual corneal concentrations and bioavailability,[27] the fluoroquinolones (ciprofloxacin and ofloxacin) tended to have the lowest overall MIC values.

Clinical Data

Completed clinical outcome data were available for 421 eyes (421 patients). Complete data were unavailable on the remaining patients due to incomplete follow-up. There was no significant difference in the proportions of the main organism groups between the patients with data available and those without (Table 1).

Risk Factors for Keratitis

The risk factors were placed into one of five groups for analysis: group 1 (32%), contact lens wear (daily wear soft, extended wear soft, or rigid gas permeable); group 2 (27%), corneal disease (edema, dystrophy, keratopathy, recurrent erosion, foreign body trauma, previous corneal surgery); group 3

TABLE 4. Clinical Characteristics and Outcome of Patients with Healed Versus Nonhealed Ulcers or Those That Required Surgery

	Ulcer Size Major Axis (mm)	Ulcer Size Minor Axis (mm)	Distance from Limbus (mm)	TT (d)	HT (d)
Surgery (*n* = 23)	4.57	3.75	2.30	35	35
Enucleation (*n* = 16)	6.19	5.01	1.98	28	37
	2.14	1.66	2.53	20	13
Healed (*n* = 382)	*P* < 0.01	*P* < 0.01	NS	*P* = 0.016	

Mean treatment (TT) and healing time (HT) in days.

(7%), systemic disease (autoimmunity, immunosuppression, diabetes, other); group 4 (23%), ocular surface disease (atopic keratoconjunctivitis, limbal stem cell dysfunction, chemical injury, blepharitis, trichiasis and topical steroids); and group 5 (11%), previous microbial keratitis (herpetic keratitis, bacterial keratitis).

Antimicrobials Used

Monotherapy was used in 84% and combination therapy in 16% of patients. A fluoroquinolone (ciprofloxacin or ofloxacin) accounted for 84% of monotherapy treatment and was also used in 9% of cases of combination therapy (Table 2). Cefuroxime (25%), chloramphenicol (22%), and teicoplanin (13%) were the most frequent agents used in combination therapy. When a change in antimicrobial therapy was made, chloramphenicol, a fluoroquinolone, cefuroxime, gentamicin, or teicoplanin were the predominant agents used.

Overall Outcome

A total of 16 (4%) patients required enucleation and a further 23 (5%) required surgical treatment. In the remaining 382 (91%), the ulcer healed with topical antimicrobial therapy. Of the 16 patients who lost their eyes, 6 (38%) had a streptococcal infection (three due to *S. pneumoniae*), 2 *S. aureus*, 2 CNS, 2 *Pseudomonas* spp., 2 *Enterobacteriaceae*, 1 *Neisseria* sp., and 1 with a *Corynebacterium* sp. Patients who had *S. pneumoniae* isolated from their corneal ulcers were significantly more likely to have lost an eye or to have needed surgical intervention than were those in the other bacterial groups ($P <$ 0.001). A fluoroquinolone had been used in 22 of the 23 patients who required surgery and in all the 16 patients who had an enucleation, either as monotherapy ($n = 14$) or in combination with teicoplanin ($n = 6$), chloramphenicol ($n = 8$), cefuroxime ($n = 8$), or penicillin ($n = 2$).

Ulcer Size and Healing Time

The mean (SD) ulcer size in the 421 patients was 2.14 (1.48) × 1.66 (1.18) mm, and the ulcer was situated 2.53 (1.31) mm from the limbus. Mean scar dimensions were 1.57 (1.46) × 1.27 (1.18) mm, giving a scar-to-ulcer area ratio of 0.74 (1.25). The relationship of ulcer size and healing time (HT/UA) for each of the major groups of bacteria is seen in Table 3. The largest ulcers occurred with *Pseudomonas* spp. and *Enterobacteriaceae* infections.

For eyes that healed without surgical intervention, the mean healing and treatment times were 13.10 (SD 14.10) and 20.44 (14.97) days, respectively (Table 4). When compared with this group, patients who needed surgical intervention (mean ulcer dimensions: 4.57 × 3.75 mm) or who lost an eye (mean ulcer dimensions: 6.19 × 5.01 mm) had significantly larger ulcers at presentation ($P < 0.01$) and significantly longer treatment times ($P < 0.01$; Table 4). There was a linear relationship between healing time and ulcer size ($R^2 = 0.22$, $P < 0.0001$). This relationship may have been limited by the adequacy of the collection of clinical data. Patients with small ulcers (for example, $< 1 × 1$ mm) were not seen daily during healing, thus skewing the healing time for small ulcers, as is evident in Table 5. This effect was reduced by using the ratio of the HT/UA, with a mean HT/UA of 3.54 (SD 2.61) days per square millimeter.

Linear Modeling of Determinants of Clinical Outcome

A general linear model was used to analyze the determinants of clinical outcome, as defined by the ratio HT/UA. Significant effects included the type of organism ($P = 0.001$), nearest

TABLE 5. Ulcer Size and Healing Time

UA (mm^2)	HT (d)	HT/UA (d/mm^2)
UA ≤ 0.5	5.14 (4.27)	18.24 (15.16)
0.5 < UA 1	6.10 (4.68)	6.56 (4.91)
UA > 1	18.26 (17.95)	3.86 (4.31)

Mean (SD) ulcer area (UA), healing time (HT) and ratio of healing time to ulcer area (HT/UA).

distance of the ulcer from the limbus ($P = 0.02$) and the MIC of the first antimicrobial used or lowest MIC if combination therapy had been used ($P = 0.006$). There was no apparent effect of referral center, sex, age, or type of risk factor ($P = 0.39$), so these factors were then excluded from the model. Because of the possible confounding effects of different antimicrobials, monotherapy, and combination therapy, the final model included only the subgroup of patients who received initial monotherapy with a fluoroquinolone (ciprofloxacin or ofloxacin), had no subsequent change in their antimicrobial treatment, and had ulcers that healed without surgical intervention. There were significant linear associations between clinical outcome and MIC (Fig. 1) for *Enterobacteriaceae* ($n = 30$, $R^2 = 0.14$, $P = 0.045$, Fig. 1A), *Pseudomonas* spp. ($n = 53$, $R^2 = 0.1$, $P = 0.047$, Fig. 1C), and *S. aureus* ($n = 29$, $R^2 = 0.14$, $P = 0.04$, Fig. 1D), but not for *Streptococcus* spp. ($n = 49$, $R^2 = 0.03$, $P = 0.85$, Fig. 1B), and CNS ($n = 84$, $P = 0.88$, Fig. 1E).

DISCUSSION

Isolation and identification of a bacterial species in microbial keratitis can guide the selection of an appropriate antimicrobial. The choice of antimicrobial is based on the susceptibility of the isolated bacteria to the available agents, but susceptibility rests on the assumption that the MIC is a significant factor in determining clinical outcome in microbial keratitis. Disc and MIC susceptibility breakpoints are based on the anticipated response of the bacteria against concentrations of the antimicrobial that can be achieved in serum.[24,25] Topical application of an antimicrobial to the cornea may, however, achieve a very different tissue concentration and bioavailability than in the serum.[27] The corneal penetration and effectiveness of a topical antimicrobial agent is dependent on the physicochemical properties of the antimicrobial and structure of the cornea.[30–33] For example, the cornea contains many small aqueous pathways for low-molecular-weight molecules, but a limited number of larger paracellular aqueous pathways through which high-molecular-weight molecules can penetrate.[30–33] Furthermore, pH and protein binding of the local environment and interaction with other chemicals differ from systemic conditions, particularly in the inflamed eye.[30–33] Topical administration of an antimicrobial to the eye involves its mixing with the tear film so that loss of drug from the precorneal area is a net effect of corneal and noncorneal absorption and tear secretion and drainage. In addition, host factors are likely to play a major role in determining outcome in microbial keratitis. The purpose of this study was therefore, to explore the relationship between the MIC of the infecting bacteria and the clinical outcome in a representative sample of patients with microbial keratitis treated in routine clinical practice in the United Kingdom.

The range of bacteria isolated in this study was similar to that reported by Tuft and Matheson[10] for 1312 isolates for the period 1984 to 1999 and for other studies from the United Kingdom and other countries.[7–9,12] The 421 completed clinical cases represents a substantial sample, including more than 50%
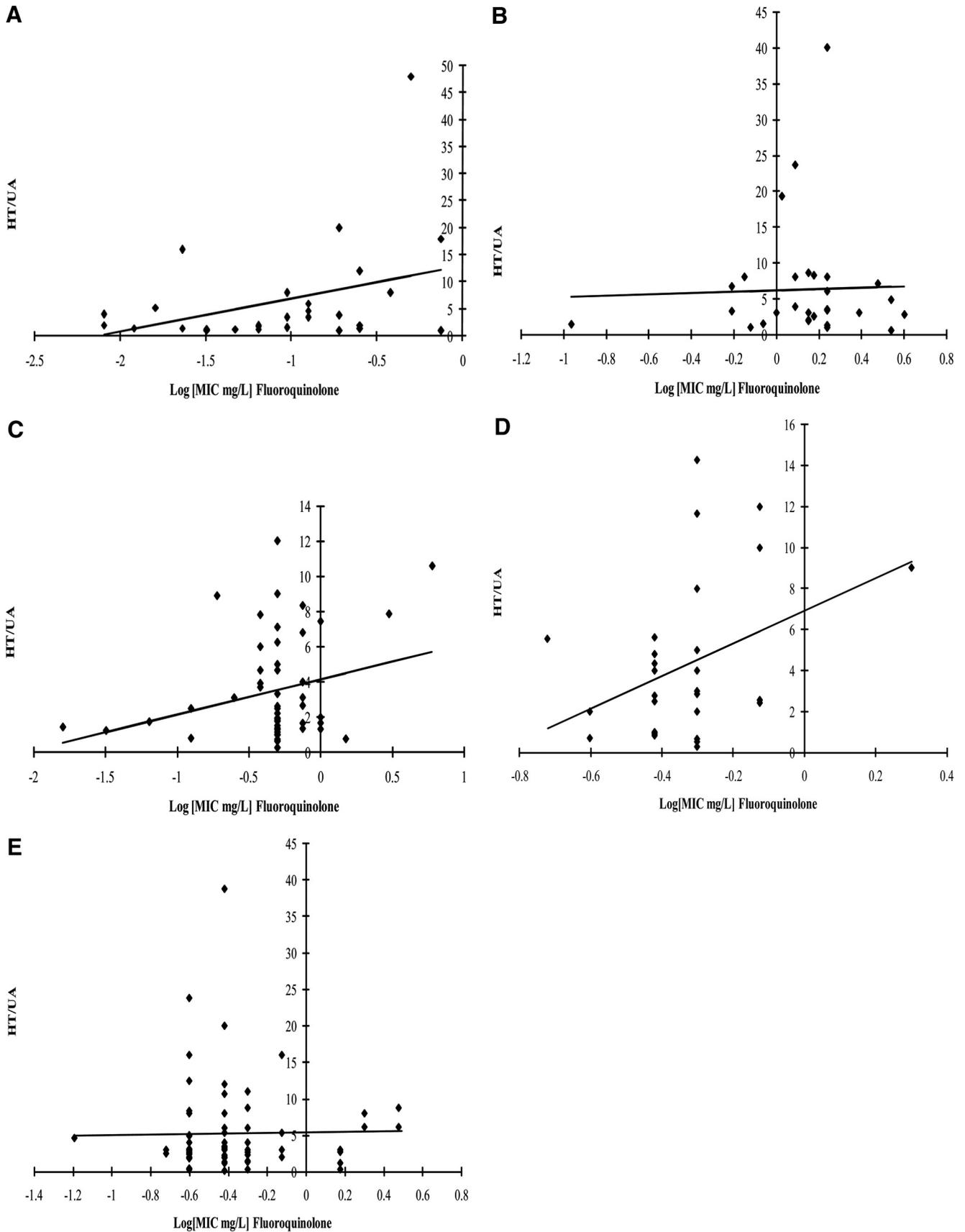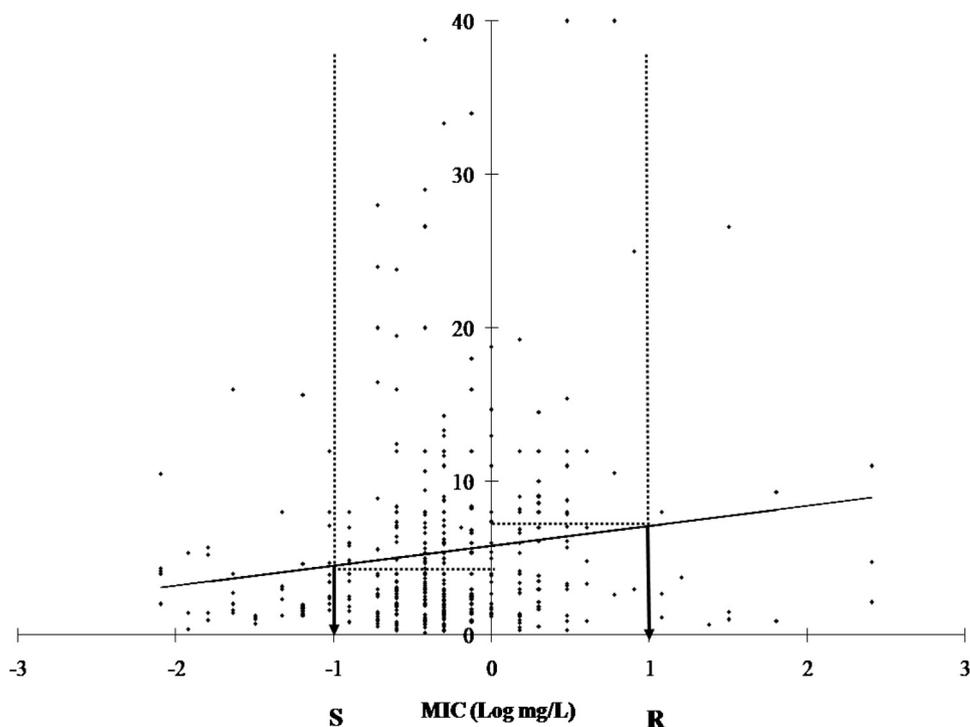
FIGURE 1.   Clinical outcome and MIC. HT/UA (days per square millimeter), logarithm (Log) of MIC. Fluoroquinolone and (**A**) *Enterobacteriaceae*, (**B**) *Streptococcus* spp., (**C**) *Pseudomonas* spp., (**D**) *S. aureus*, (**E**) CNS.

FIGURE 2. Clinical outcome and MIC: antimicrobial used and all bacterial isolates. HT/UA (in days per square millimeter), logarithm (Log) of MIC. Hypothetical susceptible and resistant outcomes (HT/UA of 3.5 days/mm^2 and 7 days/mm^2) corresponds to an MIC of 0.1 and 10 mg/L.

of the patients from whom a bacterial isolate was recovered with a representative proportion of each of the principle bacterial groups. Almost all patients had at least one risk factor with at least one third attributable to contact lens wear and a quarter each to corneal and ocular surface disease, consistent with previous reports.[2,7–9,11,13–15] Because of the heterogeneity of the data, in particular the number of risk factors, it was not possible to determine whether a particular factor—for example, topical steroid—led to an improved or worse outcome.

Consistent with previous reports, patients with larger ulcers tended to have a significantly worse outcome and a greater risk of loss of the eye. Isolation of a *Streptococcus* spp., and in particular a *S. pneumoniae*, was also more likely to be associated with loss of an eye. Ciprofloxacin and ofloxacin were the most commonly used empiric monotherapy in this study, but they are less active against streptococci such as *S. pneumoniae* than they are against Gram-negative bacteria. For patients presenting with large ulcers, especially where a *Streptococcus* spp. is suspected or detected on a smear, ciprofloxacin or ofloxacin monotherapy may be inappropriate and the addition of an antimicrobial with good activity against *Streptococcus* spp. should be considered.

The fluoroquinolones, in particular ciprofloxacin, had the lowest MIC$_{90}$ for all organisms except the streptococci. In most instances the MIC$_{90}$ concentration for these organism/antimicrobial combinations was below the systemic breakpoint, and these organisms would therefore routinely be reported as susceptible by microbiology laboratories. The median MIC for ciprofloxacin against *Pseudomonas* spp. was similar to the range (0.12–0.25 g/L) reported by Lomholt and Kilian[34] The MIC$_{90}$ for ciprofloxacin and ofloxacin were similar to those reported for streptococci, CNS, and staphylococci by Oliveira et al.[35] for corneal isolates in Brazil. For *S. pneumoniae* in the present study, penicillin, cefuroxime, and teicoplanin exhibited the lowest MIC$_{90}$s, which were all below the systemic breakpoints for this organism. The MIC$_{90}$s for the fluoroquinolones against streptococci (*S. pneumoniae* and other streptococci) suggest a reduced susceptibility of these

organisms to this group of antimicrobials. Similarly, the aminoglycosides (gentamicin and amikacin) have higher MICs against streptococci than against the other organism groups.

One of the difficulties in the treatment of microbial keratitis is the apparent absence of a predictable association between antimicrobial bacterial susceptibility and clinical outcome.[20,24,25] Wilhelmus et al.[11] reported a multicenter center study on 391 eyes of 663 patients with culture-confirmed, susceptibility-tested microbial keratitis, treated with ciprofloxacin over a 14-day period. In support of a systemic breakpoint of 1 mg/L for ocular isolates, they reported that patients in whom the principal corneal isolate had a ciprofloxacin MIC exceeding 1 mg/L improved significantly more slowly than those with a more susceptible isolate. In addition, 272 (74.5%) of patients with isolates with a ciprofloxacin MIC less than 1 mg/L had successful epithelial healing compared with 15 (57.7%) of with a less sensitive isolate.[11]

Figure 2 summarizes the relationship in the patients in this study between clinical outcome data (HT/UA) and the lowest MIC of the particular antimicrobial agent used. Such data could be used to determine an appropriate topical MIC breakpoint. If, for example, an HT/UA of 3.5 days per mm^2 was considered indicative of a satisfactory response to topical therapy and a HT/UA of 7 d/mm^2 consistent with an unacceptable response, the corresponding MICs would be 0.1 and 10 mg/L, respectively. As a concentration and bioavailability of an antimicrobial of >10 mg/L are unlikely to be achieved in the cornea,[27] this concentration might then be considered the breakpoint. Although there are technical differences in microbiology guidelines between, for example, the British Society for Antimicrobial Chemotherapy (BSAC), The European Committee on Antimicrobial Susceptibility Testing (EUCAST), and Clinical and Laboratory Standards Institute (CLSI), the approach we propose is equally applicable for all these methods. In this study, the general linear multivariate model revealed a weak but significant association between the MIC of the antimicrobial prescribed and clinical outcome defined by the ratio of healing time to ulcer size. The importance of the bacterial type

and antimicrobial used for treatment, is indicated by the significant associations between the fluoroquinolone MIC and clinical outcome for *Pseudomonas* spp., *S. aureus*, and *Enterobacteriaceae* but not for *Streptococcus* spp. or CNS. The absence of an association for CNS may reflect the importance of host factors or simply that, in a proportion of cases, this isolate was not pathogenic or was a contaminant. Evaluating the clinical significance of CNS isolates from samples is invariably difficult. Conversely, the absence of an apparent association between *Streptococcus* spp. and fluoroquinolone monotherapy may reflect the relatively poor activity of the fluoroquinolones against streptococci.[12,35]

In a prospective study, using defined treatment regimens and daily measurements of ulcer size, investigators could then use this type of approach to determine appropriate disc susceptibility and MIC breakpoints for ophthalmic isolates. Such breakpoints could then be used in ophthalmic practice for determining whether a given isolate is resistant to a given topical antimicrobial.

Acknowledgments

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