Distribution of bacterial keratitis and emerging resistance to antibiotics in China from 2001 to 2004

Chen Zhang
Yanchuang Liang
Shijing Deng
Zhiqun Wang
Ran Li
Xuguang Sun
Department of Ocular Microbiology, Beijing Institute of Ophthalmology, Beijing Tongren hospital, Capital University of Medical Science, Beijing

Correspondence: Xuguang Sun
Chairman of Eye Microbiology
Department, Beijing Institute of Ophthalmology, 17 Hou Gou Lane, Chong Nei Street, Beijing 100005, China
Tel +86 01 5826 5935
Email sunxuguang@yahoo.com

Objective: To study on the distribution of bacterial keratitis isolates and the resistance to antibiotics in China from 2001 to 2004.

Methods: 1985 specimens from the bacterial keratitis at the Beijing Tong Ren Eye Center were cultured and identified. In vitro susceptibility testing of positive isolates to antibiotics was determined by the Kirby-Bauer disk diffusion method and interpreted according to Clinical and Laboratory Standards Institute.

Results: Out of 1985 specimens, 279 were culture positive. The percentage of positive culture was 14.06%. Gram-positive cocci and gram-negative bacilli represented 42.65% (119/270) and 35.13% (98/279) respectively. *Pseudomonas* sp. was the most common organism (20.07%), followed by *Corynebacterium* sp. (16.85%) and *Staphylococcus epidermidis* (13.98%). Resistance to ofloxacin, ciprofloxacin, levofloxacin, and tobramycin was 20.2%, 35.9%, 15.5%, and 29.4% respectively. Gram-negative bacilli showed higher resistance to ciprofloxacin. *Staphylococcus* sp. revealed significant resistance to ciprofloxacin. *Streptococcus* sp. showed high resistance to tobramycin. The resistance of isolates from older patients (≥60Y) to ciprofloxacin, levofloxacin, and tobramycin was higher than that from adult patients (>14 to 59Y).

Conclusion: *Staphylococcus* sp., *Pseudomonas* sp., and *Corynebacterium* sp. were the most common bacterial keratitis isolates in China. Attentions should be paid to the increase of the resistance to levofloxacin.

Keywords: bacteria keratitis resistance

Bacterial keratitis represents one of the most common causes of corneal blindness in developing countries (Limberg 1991). Different bacterium may result in some different lesions on the cornea and present various susceptibilities to antibiotics. With the extensive application of broad-spectrum antibiotics and the change of the bacterial biological characteristics, the bacterium spectrum and susceptibility to antibiotics have also changed. It is necessary to study the distribution and emerging resistance of organism for guidance of clinical diagnosis and treatment. The purpose of this study was to review the distribution and the trends of the bacterial keratitis and the susceptibility of the corneal isolates from 2001 to 2004 in China.

Material and methods

All consecutive patients with clinical diagnosis of bacterial keratitis from January 1, 2001 through December 31, 2004 at the Beijing Tong Ren Eye Center were included. The initial corneal smears, cultures, and identification with a routine technique were investigated for all patients. The specimens were sent to the Department of Ocular Microbiology in Beijing Institute of Ophthalmology. Corneal scrapings were obtained with a platinum spatula, and then inoculated on blood-agar plate at 35–37 °C for 24–72 hrs. Bouillon culture-medium was used for special specimens when the quantity of
specimens was small before being cultured. Initial identification was made according to the characteristics of growing colonies on blood-agar plate and Gram-staining. Final identification was based on Bio-Merieux tests with identification medium and biochemical event.

Antibiotic susceptibility was determined with the Kirby-Bauer disc diffusion method and interpreted according to Clinical and Laboratory Standards Institute.

Statistical calculation was performed with the SPSS 11.5 statistical software package (SPSS Inc., Chicago, IL). The result was managed using the chi-square test. $P < 0.05$ was considered to be statistically significant difference.

Results
During the four-year period from 2001 to 2004, 1985 patients were included. Bacterial cultures were positive in 279 patients (14.06%), 151 (54.12%) in male and 128 (46.88%) in female, with a mean age of 45.20 years (range 1–96). All patients were divided into three age-groups as followed: 14 in the child group (0–14 years old), 193 in the adult group (15–59 years old), and 72 in the elderly group ($\geq$60 years old).

Distribution of bacterial keratitis isolates
Among the positive cultures, the number of Gram-positive cocci was 119 (42.65%), and *Staphylococcus epidermidis* (13.98%) was the most common organism in Gram-positive cocci. The number of Gram-positive bacilli isolates accounted for 58 (20.79%), within which *Corynebacterium sp.* (16.85%) was the most common organism. Gram-negative bacilli isolates were 98 (35.12%), and *Pseudomonas sp.* was 20.07%. Gram-negative cocci isolates were 4 (1.43%). The ratio of Gram-positive to Gram-negative was 1.74:1 (63.44%: 36.56%). Table 1 listed the distribution of isolated organisms in positive culture of isolates.

In the adult group, *Staphylococcus sp.*, *Pseudomonas sp.*, and *Corynebacterium sp.* were the most common organisms, Meanwhile *Staphylococcus sp.*, *Streptococcus sp.*, and *Corynebacterium sp.* were the most common organisms in the elderly group. The number of *Streptococcus sp.* in the elderly group was significantly higher than that in the adult group ($P < 0.01$). Distribution of the bacterial keratitis isolates in all age-groups is shown in Table 2.

\textbf{In vitro resistance to antibiotics}
Two hundred and forty-five positive isolates were involved in the resistance test and the percentage of resistance to ofloxacin, ciprofloxacin, levofloxacin, and tobramycin was 20.8%, 35.9%, 15.5%, and 29.4%, respectively (Table 3). The percentage of resistance to ciprofloxacin and tobramycin was significantly higher than that to levofloxacin ($P < 0.01$). The percentage of resistance to ciprofloxacin and tobramycin was higher than that to ofloxacin ($P < 0.05$).

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|}
\hline
\textbf{Bacteria} & \textbf{2001–2002} & & \textbf{2003–2004} & & \textbf{Total} \\
\hline
& \textbf{Isolates} & \textbf{Percent (%)} & \textbf{Isolates} & \textbf{Percent (%)} & \textbf{Isolates} & \textbf{Percent (%)} \\
\hline
*Staphylococcus epidermidis* & 22 & 15.83 & 17 & 12.14 & 39 & 13.98 \\
*Staphylococcus aureus* & 9 & 6.47 & 11 & 7.86 & 20 & 7.17 \\
*Micrococcus sp.* & 18 & 12.95 & 9 & 6.43 & 27 & 9.68 \\
*Pneumococcus* & 11 & 7.91 & 10 & 7.14 & 21 & 7.53 \\
*Streptococcus* & 3 & 2.16 & 3 & 2.14 & 6 & 2.15 \\
Other Gram-positive cocci & 2 & 1.44 & 4 & 2.86 & 6 & 2.15 \\
\hline
Total Gram-positive cocci & 65 & 46.76 & 54 & 38.57 & 119 & 42.65 \\
\hline
*Corynebacterium sp.* & 22 & 15.83 & 25 & 17.86 & 47 & 16.85 \\
*Actinomyces sp.* & 3 & 2.16 & 3 & 2.14 & 6 & 2.15 \\
*Bacillus sp.* & 3 & 2.16 & 1 & 0.71 & 4 & 1.43 \\
Other Gram-positive bacilli & 1 & 0.71 & 0 & 0 & 1 & 0.36 \\
\hline
Total Gram-positive bacilli & 29 & 20.86 & 29 & 20.71 & 58 & 20.79 \\
\hline
*Pseudomonas sp.* & 25 & 17.99 & 31 & 22.15 & 56 & 20.07 \\
*Cececa sp.* & 7 & 5.04 & 0 & 0 & 7 & 2.51 \\
*Serratia sp.* & 1 & 0.71 & 3 & 2.14 & 4 & 1.43 \\
*Proteus sp.* & 0 & 0 & 2 & 1.43 & 2 & 0.72 \\
*Klebsiella sp.* & 2 & 1.44 & 2 & 1.43 & 4 & 1.43 \\
Other Gram-negative bacilli & 8 & 5.76 & 17 & 12.14 & 25 & 8.96 \\
\hline
Total Gram-negative bacilli & 43 & 30.94 & 55 & 39.29 & 98 & 35.12 \\
Total Gram-negative cocci & 22 & 1.44 & 2 & 1.43 & 4 & 1.43 \\
\hline
Total bacterial isolates & 139 & 100 & 140 & 100 & 279 & 100 \\
\hline
\end{tabular}
\caption{Organisms in bacterial isolates during 2001–2004 in China}
\end{table}
The results in this study showed no significant difference between the resistances to four antibiotics for gram-positive bacilli ($P > 0.05$). Gram-negative bacilli showed higher resistance to ciprofloxacin than to other three antibiotics ($P < 0.01$). *Staphylococcus* sp. showed higher resistance to ciprofloxacin ($P < 0.05$). *Streptococcus* sp. had higher resistance to tobramycin ($P < 0.01$) (Table 4).

Antibiotic resistance of bacterial isolates in age-groups is shown in Table 5. The resistance to ciprofloxacin, levofloxacin, and tobramycin in the elderly group was higher than that in the adult group ($P < 0.05$). The child group was not compared with other groups as a result of the small number of isolates identified.

**Table 2** Distribution of the bacterial keratitis isolates in different age-groups

| Bacteria                  | Child group | Adult group | Elderly group |
|---------------------------|-------------|-------------|---------------|
|                           | Isolates    | Percent (%) | Isolates      | Percent (%) | Isolates    | Percent (%) |
| Staphylococcus sp.        | 4           |             | 67            | 34.7        | 19          | 26.4         |
| Streptococcus sp.         | 2           |             | 11            | 5.7         | 16          | 22.2         |
| **Total Gram-positive cocci** | 6           |             | 78            | 40.4        | 35          | 48.6         |
| Corynebacterium sp.       | 2           |             | 33            | 17.1        | 12          | 16.7         |
| Other Gram-positive bacilli | 0           |             | 10            | 5.2         | 1           | 1.4         |
| **Total Gram-positive bacilli** | 2           |             | 43            | 22.3        | 13          | 18.1         |
| Pseudomonas sp.           | 4           |             | 42            | 21.8        | 10          | 13.9         |
| Other Gram-negative bacilli | 2           |             | 27            | 14.0        | 13          | 18.0         |
| **Total Gram-negative bacilli** | 6           |             | 69            | 35.8        | 23          | 31.9         |
| Gram-negative cocci       | 0           |             | 3             | 1.5         | 1           | 1.4         |
| **Total bacterial isolates** | 14          |             | 193           | 100         | 72          | 100          |

**Discussion**

Bacterial keratitis is a devastating ophthalmologic emergency and is the most common reason leading to corneal blindness in developing countries. It is very important for clinical diagnosis and treatment to study the characteristic of distribution and resistance of bacterial isolates. Sun and colleagues (2004) reported the distribution and shifting trends of bacterial keratitis (1989–1998) and presented

**Table 3** Susceptibility tests of the keratitis isolates

| Bacteria                  | Ofloxacin | Ciprofloxacin | Levofloxacin | Tobramycin |
|---------------------------|-----------|---------------|--------------|------------|
|                           | S | I | R | S | I | R | S | I | R | S | I | R |
| *Staphylococcus epidermidis* | 24 | 0 | 15 | 18 | 8 | 13 | 27 | 4 | 8 | 23 | 3 | 13 |
| *Staphylococcus aureus*    | 16 | 0 | 4 | 11 | 5 | 4 | 17 | 0 | 3 | 13 | 0 | 7 |
| *Micrococcus sp.*          | 21 | 0 | 6 | 17 | 3 | 7 | 25 | 1 | 1 | 23 | 2 | 2 |
| *Pneumococcus*             | 17 | 0 | 3 | 13 | 3 | 4 | 16 | 0 | 4 | 4 | 0 | 16 |
| *Streptococcus ex-hem.*    | 3  | 0 | 2 | 3  | 0 | 2 | 3  | 0 | 2 | 1  | 0 | 4 |
| *Streptococcus β-hem.*     | 0  | 0 | 2 | 1  | 0 | 1 | 2  | 0 | 0 | 2  | 0 | 0 |
| Other Gram-positive cocci  | 2  | 0 | 2 | 2  | 0 | 2 | 2  | 0 | 2 | 2  | 0 | 2 |
| **Total Gram-positive bacilli** | 83 | 0 | 34 | 65 | 19 | 33 | 92 | 5 | 20 | 68 | 3 | 44 |
| *Corynebacterium sp.*      | 23 | 0 | 7 | 18 | 2 | 10 | 25 | 1 | 3 | 23 | 2 | 5 |
| *Actinomyces sp.*          | 2  | 0 | 0 | 2  | 0 | 0 | 2  | 0 | 0 | 2  | 0 | 0 |
| Other Gram-positive bacilli | 5  | 0 | 0 | 5  | 0 | 0 | 5  | 0 | 0 | 5  | 0 | 0 |
| **Total Gram-positive bacilli** | 30 | 0 | 7 | 25 | 2 | 10 | 32 | 1 | 3 | 30 | 2 | 5 |
| *Pseudomonas sp.*          | 49 | 2 | 4 | 42 | 9 | 4 | 54 | 0 | 2 | 48 | 1 | 6 |
| *Klebsiella sp.*           | 4  | 0 | 0 | 3  | 0 | 1 | 4  | 0 | 0 | 3  | 0 | 1 |
| *Serratia sp.*             | 4  | 0 | 0 | 3  | 1 | 0 | 4  | 0 | 0 | 3  | 0 | 1 |
| *Proteus sp.*              | 3  | 0 | 0 | 2  | 1 | 0 | 3  | 0 | 0 | 2  | 0 | 1 |
| Other Gram-negative bacilli | 19 | 0 | 3 | 15 | 4 | 3 | 18 | 0 | 4 | 15 | 1 | 6 |
| **Total Gram-negative bacilli** | 79 | 2 | 7 | 65 | 15 | 8 | 83 | 0 | 6 | 71 | 2 | 15 |
| Gram-negative cocci        | 2  | 1 | 0 | 2  | 1 | 0 | 3  | 0 | 0 | 3  | 0 | 0 |
| **Total**                 | 194 | 3 | 48 | 157 | 37 | 51 | 210 | 6 | 29 | 172 | 9 | 64 |

**Abbreviations:** R, resistant; I, intermediate; S, sensitive; I-R, percentage of resistance to antibiotic strain; S+I-R, strain $\times 100\%$.
22.1% positive culture rate and the trend of increase in Gram-positive bacterium (51.0%, of the total positive culture) with the decrease in Gram-negative bacterium (39.4%, of the total positive culture). The rate of *Pseudomonas sp.* and Gram-positive bacilli was 31.90% and 20.07%, respectively. Alexandrakis and colleagues (2000) studied the corneal specimens from 1990 to 1998 and reported 50% positive culture rate and the trend of increase in Gram-positive keratitis isolates (48.0%, of the total positive culture) with a corresponding decrease in Gram-negative organisms (50%, of the total positive culture), and the percentage of *Pseudomonas sp.* was 25.7% in their report. In this study, the rate of positive culture was 14.15%, with Gram-positive cocci 42.65%, Gram-negative bacilli 35.12%, and Gram-positive bacilli 20.79%. Compared with our previous study (Sun et al 2004), Gram-negative bacilli and Gram-positive cocci were still the main organisms in bacterial keratitis and *Pseudomonas sp.* remained the most common organism in China.

Butler and colleagues (2005) retrospectively analyzed four-year data on bacterial keratitis in older patients. They found that Gram-positive cocci was 75.9% and Gram-negative bacilli was 25.9%. In this study, the percentage of Gram-positive cocci in older patients (48.6%) was slightly higher than that in adult patients (40.4%). The number of *Streptococcus sp.* in elderly patients (22.2%) was obviously higher than that in adult patients (5.7%).

With the extensive application of broad-spectrum antibiotics, the rate of resistance to antibiotics has increased gradually in China. Sun and colleagues (2003) reported that during 1999 to 2000, the percentage of resistance to ofloxacin, ciprofloxacin, and norfloxacin was 28.5%, 25.9%, and 34.9%, respectively. Alexandrakis and colleagues (2000) demonstrated that there was a trend of gradual increase in the resistance for *Staphylococcus aureus* from 1990 to 1998. The resistance to quinolone and aminoglycosides for *Staphylococcus aureus* was 15% and 11%, respectively. However, the resistance of *Pseudomonas aeruginosa* was 1% and 0.6%, respectively. Goldstein and colleagues (1999) studied the resistance of 1053 bacterial keratitis isolates in vitro from 1993 to 1997 and found that the resistance to ciprofloxacin and ofloxacin was 16.9% and 14.3%, respectively. The previous study in China showed that the percentage of resistance to ofloxacin, ciprofloxacin, levofloxacin, and tobramycin was 23.6%, 35.7%, 4.1%, and 26.0% in 1999. This study showed that the total resistance to ofloxacin (20.8%), ciprofloxacin (35.9%), and tobramycin (29.4%) was similar to the previous studies. But the resistance to levofloxacin (15.5%) of bacterial keratitis isolates has

### Table 4. Antibiotic resistance of keratitis isolates

| Bacteria             | Total isolates | Ofloxacin Resistance isolates | % | Ciprofloxacin Resistance isolates | % | Levofloxacin Resistance isolates | % | Tobramycin Resistance isolates | % |
|----------------------|---------------|-------------------------------|---|----------------------------------|---|----------------------------------|---|-------------------------------|---|
| *Staphylococcus sp.* | 59            | 19                            | 32.2% | 30                              | 50.8% | 15                              | 25.4% | 23                            | 39.0% |
| *Streptococcus sp.*  | 27            | 7                             | 25.9% | 10                              | 37.0% | 6                               | 22.2% | 20                            | 74.1% |
| Gram-positive bacilli| 37            | 7                             | 18.9% | 12                              | 32.4% | 5                               | 13.5% | 7                             | 18.9% |
| Gram-negative bacilli| 88            | 9                             | 10.2% | 23                              | 26.1% | 6                               | 6.8%  | 17                            | 19.3% |
| Gram-negative cocci  | 3             | 1                             | 3.5%  | 1                               | 3.5%  | 0                               | 0     | 0                             | 0     |
| Total                | 245           | 51                            | 20.8% | 88                              | 35.9% | 38                              | 15.5% | 72                            | 29.4% |

**Notes:** *Staphylococcus sp.* mainly include *Staphylococcus epidermidis,* or *Staphylococcus aureus.*

### Table 5. Antibiotic resistance of keratitis isolates in age-groups

| Bacteria | Total isolates | Ofloxacin Resistance isolates | % | Ciprofloxacin Resistance isolates | % | Levofloxacin Resistance isolates | % | Tobramycin Resistance isolates | % |
|----------|---------------|-------------------------------|---|----------------------------------|---|----------------------------------|---|-------------------------------|---|
| Children | 13            | 1                             | 7.7% | 2                               | 15.4% | 0                               | 0%  | 5                             | 38.5% |
| Adults   | 267           | 36                            | 13.5% | 57                              | 21.3% | 25                              | 9.4% | 41                            | 15.4% |
| Elderly  | 65            | 14                            | 21.5% | 29                              | 44.6% | 13                              | 20.0% | 26                            | 40.0% |
| Total    | 245           | 51                            | 20.8% | 88                              | 35.9% | 38                              | 15.5% | 72                            | 29.4% |
Distribution of bacterial keratitis and emerging resistance to antibiotics in China

gradually increased. And the resistance of gram-positive cocci to antibiotics was much higher than that of gram-negative bacilli. The resistance to ciprofloxacin, levofloxacin, and tobramycin in older patients was much higher than that in adult patients.

In summary, *Pseudomonas sp.* and *Staphylococcus sp.* were the most common organisms in bacterial keratitis in China. The percentage of *Pseudomonas sp.* and *Staphylococcus sp.* in the older patients was lower than that in adults, while the percentage of *Streptococcus sp.* was higher. There was a trend of increase in the resistance to levofloxacin.

**References**

Alexandrakis G, Alfonso EC, Miller D. 2000. Shifting trends in bacterial keratitis in south Florida and emerging resistance to fluoroquinolones. *Ophthalmology*, 107:1497–502.

Butler TK, Spencer NA, Chan CC, et al. 2005. Infective keratitis in older patients: a 4 year review, 1998–2002. *Br J Ophthalmol*, 89:591–6.

Goldstein MH, Kowalski RP, Gordon YI. 1999. Emerging fluoroquinolone resistance in bacterial keratitis: a 5-year review. *Ophthalmology*, 106:1313–18.

Limberg MB. 1991. A review of bacterial keratitis and bacterial conjunctivitis. *Am J Ophthalmol*, 112:2S–9S.

Sun X, Deng S, Li R, et al. 2004. Distribution and shifting trends of bacterial keratitis in north China (1989–98). *Br J Ophthalmol*, 88:165–6.

Sun XG, Wang ZQ, Li R, et al. 2003. In vitro fluoroquinolone resistance in ocular bacterial isolates. *Zhonghua Yan Ke Za Zhi*, 39:163–6.
