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

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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. *Staphycoccus sp.* revealed significant resistance to ciprofloxacin. S*treptococcus sp.* showed high resistance to tobramycin. The resistance of isolates from older patients (≥60Y) to ciproloxacin, 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

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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 yeas old), 193 in the adult group (15–59 years old), and 72 in the elderly group (≥60 years old).

Distribution of bacterial keratitis isolates

Among the positive cultures, the number of Gram-positive cocci was 119 (42.65%), and *Staphylococcus epdiermidis.* (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.

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 ($P < 0.05$).

Table 1 Organisms in bacterial isolates during 2001–2004 in China

Bacteria	2001–2002		2003–2004		Total	
	Isolates	Percent (%)	Isolates	Percent (%)	Isolates	Percent (%)
Staphylococcus epdiermidis	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
Pneumonococcus	11	7.91	10	7.14	21	7.53
Streptococcus α-hem.	3	2.16	3	2.14	6	2.15
Other Gram-positive cocci	2	1.44	4	2.86	6	2.15
Total Gram-positive cocci	65	46.76	54	38.57	119	42.65
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
Total Gram-positive bacilli	29	20.86	29	20.71	58	20.79
Pseudomonas sp.	25	17.99	31	22.15	56	20.07
Cedecea 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
Total Gram-negative bacilli	43	30.94	55	39.29	98	35.12
Total Gram-negative cocci	2	1.44	2	1.43	4	1.43
Total bacterial isolates	139	100	140	100	279	100

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

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.

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 epdiermidis	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
Pneumonococcus	17	0	3	13	3	4	16	0	4	4	0	16
Streptococcus α-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	5	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 × 100%.

Table 4 Antibiotic resistance of keratitis isolates

Bacteria	Total isolates	Ofloxacin		Ciprofloxacin		Levofloxacin		Tobramycin	
		Resistance isolates	%	Resistance isolates	%	Resistance isolates	%	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		1		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*.

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 5 Antibiotic resistance of keratitis isolates in age-groups

Bacteria	Total isolates	Ofloxacin		Ciprofloxacin		Levofloxacin		Tobramycin	
		Resistance isolates	%	Resistance isolates	%	Resistance isolates	%	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%

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.

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