·Clinical Research·

Bacterial spectrum and resistance patterns in corneal infections at a Tertiary Eye Care Center in South China

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Abstract

• AIM: To investigate the spectrum and antibiotic susceptibility of bacteria isolated from patients with suspected corneal infections in Zhongshan Ophthalmic Center in South China over the past four years retrospectively.

• METHODS: Totally 1943 corneal scrapes from patients with corneal infections from 2010 to 2013 were cultured and processed using standard microbiological procedures to identify bacterial isolates. Simultaneously, the bacterial isolates were tested for antibiotic susceptibility to 8 antibiotics (ceftazidime, cefuroxim, cefazolin, levofloxacin, ofloxacin, neomycin, tobramycin, chloramphenicol) using the Kirby-Bauer disc diffusion technique.

• RESULTS: Of the total 1943 scrapes, 397 (20.43%) were culture-positive, of which 294 (74.06%) were gram-positive (GP) and 103 (25.94%) were gram-negative (GN) bacteria. Of the GP organisms, the most prevalent genera were Staphylococcus spp. (56.17%, *n*=223), Kocuria spp. (5.29%, *n*=21) and Micrococcus spp. (1.26%, *n*=5). On the other hand, the most prevalent genera were Pseudomonas spp. (12.85%, *n*=51), Burkholderia spp. (2.02%, *n*=8) and Acinetobacter spp. (1.51%, *n*=6) for the GN organisms. Among five antibiotics that have eye drop products, the resistant to neomycin of GP (7.82%, 95% CI: 4.72%-10.92%) and GN isolates (9.71%, 95% CI: 4.01%-15.41%) was lowest, while the resistant to chloramphenicol was highest (GP: 34.35%, 95% CI: 28.92%-39.78%; GN: 60.19%, 95% CI: 50.74%-69.64%).

• CONCLUSION: Staphylococcus spp. was the most common bacterial pathogens isolated from patients with corneal infections in this setting. High percentages of GP and GN bacteria were mostly susceptible to neomycin and highly resistant to chloramphenicol.

• KEYWORDS: antibiotic susceptibility; epidemiology; corneal infections; bacterial spectrum

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INTRODUCTION

The prevalence of corneal bacterial infections is common among various corneal infections. Bharathi *et al* [1] and Srinivasan *et al* [2] reported that the prevalence of corneal bacterial infections among corneal microbial infections was 32.77% and 33.2% respectively. Patients may sometimes develop refractory disease or even vision loss. In clinical practice, medical treatments of corneal bacterial infections are usually initiated prior to pathogen identification and the antibiotic susceptibility test[3]. There have been many reports on the microbial spectrum of corneal infections, and the results vary case by case. A retrospective study revealing the distribution of bacterial keratitis in North China reported that, among 490 mono-bacterial positive cultures (isolated from 2220 cases), gram-positive (GP) cocci were the leading causative organism of bacteria keratitis (S. epidermidis, Micrococcus spp., S. aureus), followed by the gram-negative (GN) bacilli (Pseudomonas spp., Acinetobacter spp., Moraxella spp.)[4]. In Australia, GP bacteria (29% of scrapes) were also the most common group of organisms isolated from keratitis, most of which were GP cocci in the Staphylococcus and Streptococcus genera [5]. In the original reports from India and Brazil, the most common pathogens isolated from bacterial keratitis were various species of Staphylococcus spp. (64.5%, 51.7%, respectively) [6-7]. However, in South India, the most prevalent bacteria isolated from bacterial keratitis cases was Streptococcus pneumonia (35.95%) [1]. These different results have been attributed to the region and environment, as well as seasonal changes[5,8]. In fact, many studies have examined the types of bacteria that can be routinely cultured from swabs of ocular surface even immediately after birth, such as S .epidermidis, S. aureus, Propionibacterium, *etc* [9-10]. Although some of these organisms are normal regional flora in ocular surface, when host defenses are breached, they can be pathogenic.

Although effective antibiotics reduce the incidence of corneal bacterial infections and improve its prognosis, the unreasonable use of various antibiotics leads to the emergence of drug-resistant strains and even induce opportunistic infections caused by bacteria that are usually harmless or of low virulence [11]. Recently, a WHO report emphasized that "resistance to common bacteria has reached alarming levels in many parts of the world indicating that many of the available treatment options for common infections in some settings are becoming ineffective" (www.who.int). Antimicrobial resistance (AMR) is an issue not only for systemic diseases but also ocular infections. To date, various drug resistances in ocular infections were reported by different scholars. Shimizu *et al* [12] investigated the trend in the emergence of levofloxacin-resistant (LVFX- resistant) strains from patients with ocular infections from 2006 to 2009 in Japan, the result indicated that LVFX-resistant strains accounted for 40 out of a total of 122 strains (32.8%). Fortunately, a report from the US, Miller *et al* [13] revealed that besifloxacin may offer extended coverage for some ocular pathogens those are resistant to current fluoroquinolones. In Brazil, a study about the shifting trends *in vitro* antibiotic susceptibilities for corneal scrapes during a period of 15y demonstrated that the susceptibility to amikacin and neomycin was improved (88%-95% and 50%-85%, respectively) [14]. Based on the results above, the diversity of pathogens in ocular infections or even a change in the environment may contribute to differences in drug resistance [15]. On the other hand, multidrug resistant (MDR) bacteria has been recently re-defined as that organisms are resistant to at least one agent in each of three or more antimicrobial categories [16]. Under this new standard, the MDR bacteria profile of cornea infection has not yet been reported.

The bacterial spectra and their antibiotic susceptibility pattern of the cornea infections vary in different geographical areas, which will influence the selection of appropriate empirical treatment before laboratory microbiological reports are available in clinical practice. In order to understand the pathogenic bacterial spectrum of corneal infections and their antibiotic resistance in South China, this study retrospectively investigated and analyzed the ocular isolates obtained from clinical patients and assessed the *in vitro* susceptibility of the most common bacterial isolates to several antibiotics in an attempt to provide guidance for clinical management.

SUBJECTS AND METHODS

A retrospective review was carried out on all patients with suspected corneal bacterial infections presenting at Zhongshan Ophthalmic Center, Guangzhou, Guangdong Province, China, between January 2010 and December 2013. This study was conducted in compliance with the principles of the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Zhongshan Ophthalmic Center, Sun Yat-sen University.

Bacterial Isolation and Identification Patients with suspected corneal bacterial infections with epithelial damage or ulcers were recruited by ophthalmologists to perform a corneal scrape for smear and culture. Specimens were collected under topical anesthesia (0.5%, proparacaine hydrochloride), complying with the principle of aseptic technique, by using standard corneal scraping kits made of plates and slides that were all directly inoculated. For each patient, a portion of the corneal scraping material was used for gram-staining immediately, while the remaining sample was inoculated in nutrient broth and incubated overnight at 35℃. Subsequently, the broth was inoculated onto potato dextrose agar for fungal culture or sheep blood agar for bacterial culture. The cultures were considered positive if colonies grew at the sites of inoculation on one or more agar plates and were identified using an automated microbiology system (Vitek2 compact, BioMerieux, Inc.100 Rodolphe Street, Durham, USA). Cultures that grew fungus only were excluded.

Antibiotic Susceptibility Test Antibiotic susceptibility testing of isolated bacteria was performed *in vitro* on ceftazidime (30 μg), cefuroxim (30 μg), cefazolin (30 μg), levofloxacin (5 μg), ofloxacin (5 μg), neomycin (30 μg), tobramycin (10 μg), and chloramphenicol (30 μg) using the Kirby-Bauer disc diffusion method. Bacterial susceptibilities were recorded as "resistant", "intermediate" and "sensitive", for the purpose of the study, "intermediate" and "sensitive" were both considered "sensitive". The antibiotic susceptibility was determined in accordance with the methods of the Clinical and Laboratory Standards Institute (CLSI).

Statistical Analysis The statistical analysis was performed using SPSS 17.0 (Chicago, IL, USA). The Chi-square test was employed for the comparison of categorical variables. Differences were considered significant at $P<0.05$.

RESULTS

A total of 1943 scrapes from the suspected corneal bacterial infections were cultured at our institution during the study period. Of the 1943 samples collected, bacteria were cultured from 397 samples. Of these, the most prevalent organisms were GP organisms (74.06%, n=294), wherein the most prevalent bacterial genera were Staphylococcus spp. (56.17%, n=223), Kocuria spp. (5.29%, n=21) and Micrococcus spp. (1.26%, n=5). The GN organisms accounted for approximately 25.94% (n=103) of all isolates. Of these, the most prevalent bacterial genera were Pseudomonas spp. (12.85%, n=51), Burkholderia spp. (2.02%, n=8) and Acinetobacter spp. (1.51%, n=6). The bacterial spectrum is described in Table 1 in detail.

A comparison of the susceptibilities of GP and GN bacteria to eight antibiotics, *e.g.* ceftazidime, cefuroxim, cefazolin, levofloxacin, ofloxacin, neomycin, tobramycin and chloramphenicol, belonging to four categories, is shown in

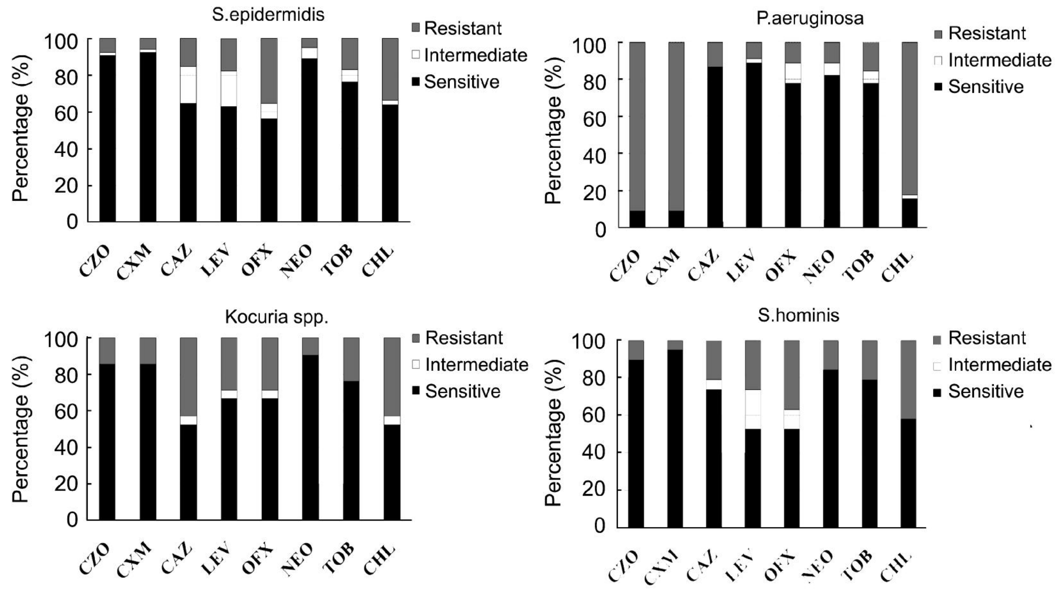

Figure 1 Bar charts showing the susceptibility of the main germs of our study namely S. epidermidis, P. aeruginosa, Kocuria and S. hominis OFX: Ofloxacin; NEO: Neomycin; CHL: Chloramphenicol; LEV: Levofloxacin; TOB: Tobramycin; CZO: Cefazolin; CXM: Cefuroxim; CAZ: Ceftazidime.

Table 1 Bacterial isolates recovered from patients with bacteria keratitis

Bacterium	*n*	%
GP organisms	294	74.06
Staphylococcus spp.	223	56.17
Kocuria spp.	21	5.29
Micrococcus spp.	5	1.26
Bacillus spp.	5	1.26
Enterococcus spp.	4	1.01
Corynebacterium spp.	4	1.01
Streptococcus spp.	3	0.76
Aerococcus spp.	3	0.76
Others GP organisms	26	6.55
GN organisms	103	25.94
Pseudomonas spp.	51	12.85
Burkholderia spp.	8	2.02
Acinetobacter spp.	6	1.51
Escherichia spp.	5	1.26
Enterobacter spp.	5	1.26
Serratia spp.	4	1.01
Chryseobacterium spp.	3	0.76
Other GN organisms	21	5.29

Table 2. Generally, the total isolates were susceptibility to quinolones, aminoglycosides and third generation of cephalosporins (*i.e.* ceftazidime). Among the five antibiotics associated with eye drop products (*i.e.* levofloxacin, ofloxacin, neomycin, tobramycin and chloramphenicol), the resistant to neomycin of GP (7.82%, 95% CI: 4.72-10.92) and GN isolates (9.71%, 95% CI: 4.01-15.41) was lowest, while the resistant to chloramphenicol was highest (GP: 34.35%, 95% CI: 28.92-39.78; GN: 60.19%, 95% CI: 50.74-69.64). Specifically, for five antibiotics that have eye drop products, three predominant GP bacteria (S. epidermidis, S. hominis and Kocuria spp.) showed a high level of susceptibility to neomycin (94.96%, 84.21%, 90.48%, respectively), followed by tobramycin (83.19%, 78.95%, 76.19%, respectively). S. epidermidis was more susceptible to neomycin than tobramycin (P=0.004). The predominant GN bacteria (P. aeruginosa) showed a high level of susceptibility to levofloxacin (91.11%), followed by neomycin (88.89%) and tobramycin (84.45%). The susceptibilities of the four main bacteria to above eight antibiotics are displayed in Figure 1.

The susceptibility of bacteria to two combined antibiotics were analyzed to explore if they produce a stronger effect in combination than either drug alone, or levofloxacin, which is widely used nowadays (Figure 2). For total bacterial isolates or GP isolates, the susceptibility to combination of tobramycin with cefazolin, cefuroxim or ceftazidime was significant higher than using either one of them or levofloxacin alone (P<0.05, Figure 2A, 2B). However, for GN isolates, the susceptibility to combination of tobramycin and ceftazidime was higher than only one drug was used (Figure 2C).

Additionally, MDR bacteria species were found in this study. We found sixty-one (15.37%) MDR bacteria those were resistant to at least one agent in each of three or more antimicrobial categories (in our study, cephalosporins, quinolones, aminoglycosides and phenicols) of antibiotics. Of these, the first-two high proportion of resistant bacteria were S. epidermidis (10.92%, 13/119) and P. aeruginosa (20%, 9/45) (Table 3).

DISCUSSION

The objective of this study was to perform a comprehensive

Table 2 The percentage of strains resistant to antibacterial agents (95% CI)

Organism	Cephalosporins			Quinolones		Aminoglycosides		Chloramphenicol
	Cefazolin	Cefuroxim	Ceftazidime	Levofloxacin	Ofloxacin	Neomycin	Tobramycin	
GP (294)	10.88 (7.37-14.43)	9.52 (6.17-12.87)	22.79 (17.99-27.59)	18.37 (13.94-22.80) [a]	27.9 (22.76-33.04)[a]	7.82 (4.72-10.92)	17.35 (13.07-21.73)[a]	34.35 (28.92-39.78)[a]
GN (103)	75.73 (67.46-84.00)	70.87 (62.09-79.65)	15.53 (8.53-22.53)	15.53 (8.53-22.53)	16.50 (9.33-23.67)	9.71 (4.01-15.41)	25.24 (16.85-33.63)[b]	60.19 (50.74-69.64)[b]
Total (397)	27.71 (23.30-32.12)	25.44 (21.25-29.83)	20.91 (16.91-24.91)	17.63（13.89-21.37）[a]	24.94 (20.69-29.19)[a]	8.31（5.59-11.03）	19.40 (15.52-23.28)[a]	41.06 (36.22-45.90)[a]

[a]*P*<0.01 *vs* neomycin (for total bacteria and GP isolates); [b]*P*<0.01 *vs* neomycin (for GN isolates).

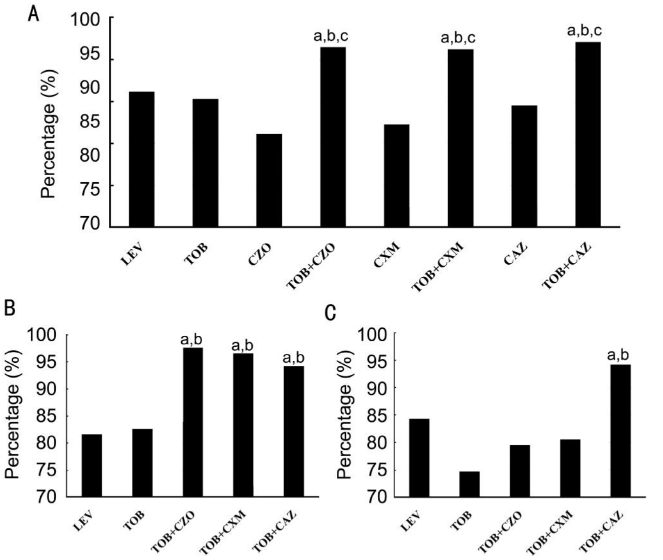

Figure 2 Comparison of susceptibilities of isolated bacteria to various combinations of antibiotics A: The susceptibility of total bacterial isolates to different combinations of antibiotics; B: The susceptibility of GP isolates to different combinations of antibiotics; C: The susceptibility of GN isolates to different combinations of antibiotics. [a,b,c]*P* <0.05, [a]: *vs* LEV; [b]: *vs* TOB; [c]: *vs* cephalosporins. LEV: Levofloxacin; TOB: Tobramycin; CZO: Cefazolin; CXM: Cefuroxim; CAZ: Ceftazidime.

Table 3 The species and numbers of multidrug resistance bacteria

Organisms (total number[b])	Positive number[a]	%
S.epidermidis (119)	13	10.92
P.aeruginosa (45)	9	20.00
B.cepacia (8)	6	75.00
S.hominis (19)	4	21.05
E.coli (5)	3	60.00
S.aueicularis (14)	3	21.43
K.roesus (11)	3	27.27
S.simulans (17)	3	17.65
S.haemolyticus (15)	3	20.00
S.warneri (14)	3	21.43
E.faecalis (2)	2	100.00
A.junii (2)	2	100.00
A.baumannii (2)	1	50.00
K.varians (3)	1	33.33
P.putida (2)	1	50.00
Methylobacterum spp. (2)	1	50.00
K.kristinas (7)	1	14.29
P.stutzeri (2)	1	50.00
E.cloacae (3)	1	33.33
Total (292)	61	20.89

[a]The positive number of the multidrug resistance; [b]The total number of each bacteria.

investigation of the bacteria causing corneal infections and their antibiotic resistance in a Tertiary Eye Hospital in South China. In our present study, the culture-positive rate in patients with suspected corneal infections was 20.43% , which approached the rate of 22.07% reported by Sun, who retrospectively investigated the distribution and shifting trends of bacterial keratitis in north China over a span of ten years[4]. In contrast, the culture positivity rates reported from Australia [17] and France [18] were 62.8% and 68.0% , respectively. Because our institution is a tertiary ocular hospital, it is likely that most of the patients received antibiotic treatment prior to the culture. Moreover, the use of topical anesthetic drops has been reported to have antibacterial effects with 24h of incubation [19-20]. These reasons may lead to our relatively low culture positivity rate.

Our results also demonstrated that the prominent pathogenic bacteria are GP bacteria, wherein Staphylococcus spp. were the most frequently isolated species (56.17%), a figure similar to that reported in Beijing[4], India[6] and Australia[5]. In Brazil, similarly, the most common pathogens (Staphylococcus spp) of bacterial keratitis accounted for 51.7% [7]. However, in the studies from Western Gujarat (India) [21] and Hong Kong [22], P. aeruginosa was the most common organism isolated. Environmental influences, the number of contact lens-related keratitis cases or the severity of cases included in each study may contribute to these differences [6]. Besides, a number of the most prevalent bacterial genera isolated from the corneal scrapings (*i.e.* Staphylococcus spp.) are opportunistic pathogens, which can cause ocular infections when host defenses are breached.

The emergence of antibiotic-resistant ocular isolates has long been a concern. In our study, 8 antibiotics (of which 5 are associated with commercial eye drops) belonging to four categories (cephalosporins, quinolones, aminoglycosides, and phenicols) were tested for resistance. Apart from cephalosporins (without eye drops), eye drops of quinolones (levofloxacin, ofloxacin) and aminoglycosides (neomycin, tobramycin) are the main products in the market, while chloramphenicol is an outdated product in China [23-24]. In the present study, our results revealed that both the GP and GN microorganisms were highly susceptible to neomycin even more than tobramycin (*P*<0.01) and highly resistant to chloramphenicol. Both neomycin and chloramphenicol were developed in the 1940s. Neomycin, which is not frequently or routinely used systemic, showed high susceptibility during the study period. However, chloramphenicol as an eye drop was widely used in Chinas a broad-spectrum antibacterial agent and was also widely used in aquaculture and animal husbandry [25]. These uses may increase the concentration of chloramphenicol residues and promote the development and abundance of bacterial resistance by spreading chloramphenicol resistance genes in the ecosystem [26]. This was supported by findings from India, Australia and London[6,27-28].

Results of systematic review and Meta-analysis suggested that fluoroquinolones may be the first choice for empirical treatment in most cases of suspected bacterial keratitis [29]. Several eye drops containing fluoroquinolones are commercially available in China. Of them, ofloxacin and levofloxacin are the most widely used [23]. Our current data revealed that the susceptibility of levofloxacin for total bacteria was up to 80%, which is higher than that of ofloxacin, and lower than that of neomycin. Among the eight antibiotics, neomycin has the lowest resistance for total isolates in this study. The ocular products of neomycin, including compound preparation (*e.g.* with polymyxin B, gramicidin or corticoid), are produced in solution or ointment form and widely used internationally [30]. It is worth noting that neomycin has nephrotoxicity, ototoxicity and causing contact dermatitis [31], which may lead to less use in systemic diseases. However, according to literature and our present data, ocular preparations of fluoroquinolones as well as neomycin are both suitable for the empirical treatment of suspected bacterial keratitis.

Combined use of antibiotics can expand the antibiotic spectrum and is already applied widely in the empirical treatment for suspected infection disease [32-33]. Our results suggested that the combined use of cephalosporin with tobramycin showed higher susceptibility for bacterial isolates, than using levofloxacin or tobramycin alone, especially for the GP bacteria. For GN isolates in our study, the P. aeruginosa accounted for the biggest proportion, which may lead to higher susceptibility of the combination of tobramycin with ceftazidime[34]. However, the side-effects of the combination therapy, particularly with tobramycin-cefazolin, were reported to be an increased risk of ocular discomfort and chemical conjunctivitis as well as a retardation effect of the epithelial-healing rate (aminoglycosides)[35-36]. Despite these, considering the higher susceptibility, the systemic or intraocular application administration is necessary when suppurative endophthalmitis occurs[37-38].

According to the new definition [16], MDR bacteria to eight antibiotics was observed in 15.4% of the isolates from cornea infection in the present study. It was emphasized that MDR of P. aeruginosa and A. baumannii became great burden pathogens, frequently being related to the high use of broad spectrum antibiotics and previous inadequate empirical antimicrobial treatment [39]. The damage of these bacteria to eyes is serious, and the clinical treatment is difficult. Additionally, it should be noted that the resistance found in vitro does not always correlate with resistance *in viva*.

In summary, we found that the most prominent pathogens in corneal bacterial infections are Staphylococcus spp., followed by P. aeruginosa. In the comparison of eight antibiotics, neomycin, levofloxacin and tobramycin may be a better choice for empirical treatment; chloramphenicol, which is widely used in ocular medicine, as well as in aquaculture and animal husbandry showed the highest resistance (41.06%) for pathogens isolated from corneal infections, indicating that chloramphenicol should not be routinely used for corneal infection in China. There is no doubt that antibiotic resistance should be taken into account in empirical treatment, and antibiotic susceptibility testing in all cases of ocular infections is essential.

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