

ORIGINAL ARTICLE

BACTERIOLOGY AND RISK FACTORS OF BACTERIAL KERATITIS IN JIMMA, SOUTHWEST ETHIOPIATewelde Tesfaye Gebremariam¹**ABSTRACT**

Background: In East Africa, particularly in Ethiopia, bacterial keratitis is a major cause of blindness.

Objective: The aims of this study were to identify risk factors of bacterial keratitis and the spectrum of bacterial etiologies, and to assess the *in-vitro* antimicrobial susceptibility of these bacterial isolates at Jimma University Specialized Hospital, Southwest Ethiopia.

Methods: A prospective study was employed from January 2012 to June 2012 from which a total of 24 patients with bacterial keratitis were included in the study. Corneal scrapings were collected, transported and microbiologically processed using standard operating procedures.

Results: Four different predisposing factors for bacterial keratitis were identified: corneal trauma 9 (37.5%), blepharitis 7 (29.2%), herpetic keratitis, and use of contaminated medications 4. Bilateral corneal infection was found in 5 (20.8%) of the cases. A total of 24 corneal scrapings were collected for microbiological evaluation, of which 20 (83%) had bacterial growth. The isolated bacterial pathogens were *Pseudomonas aeruginosa* 10 (41.7%), *Staphylococcus aureus* 5 (20.8%), *Serratia marcescens* 3 (12.5%), followed by *Streptococcus pneumoniae* 2 (8.3%). Antimicrobial susceptibility testing revealed that 85% of Gram-negative bacilli were susceptible to gentamicin and ciprofloxacin, while 86% of Gram-positive cocci were susceptible to vancomycin and Ciprofloxacin.

Conclusions: Corneal trauma was the most common risk factor for bacterial keratitis followed by blepharitis. Bacteriological analysis of corneal scrapings also revealed that *P. aeruginosa* was the most common isolate followed by *S. aureus*; the antibiotic with the highest susceptibility was ciprofloxacin. As drug resistance among bacterial pathogens is an evolving process, routine surveillance and monitoring studies should be conducted to provide an update and most effective empirical treatment for bacterial keratitis.

Keywords: Antibacterial susceptibility, Bacterial pathogens, corneal trauma, Bacterial Keratitis, Jimma, Ethiopia

INTRODUCTION

Bacterial keratitis is a major cause of preventable monocular morbidity and blindness globally particularly in East Africa. [1-3] The severity usually depends on the underlying condition of the cornea and the pathogenicity of the infecting bacteria. Hence, bacterial keratitis is an ophthalmic emergency that needs immediate treatment. [4, 5] However, antibiotic resistance among ocular pathogens is increasing worldwide. [6] Resistance increases the risk of treatment failure with potentially serious consequences. [7]

Bacterial keratitis is rare in the absence of predisposing factors. [8] Factors which influence the etiology and pathogenesis of bacterial keratitis vary. [9] Until recently, most cases of bacterial keratitis were associated with ocular trauma or ocular surface diseases. However, the widespread use of contact lenses has

dramatically increased the incidence of contact lens related keratitis. [9, 10] Moreover, the pattern of risk factors predisposing to bacterial keratitis varies with geographical regions. [9]

A wide range of bacteria can cause bacterial keratitis. However, *Pseudomonas aeruginosa* is the most frequent and the most pathogenic ocular pathogen which can cause corneal perforation in less than 24 hours after onset. [9] In addition, the bacteriological profile and their susceptibility as well as resistant patterns vary from place to place and in the same place from time to time. [5, 6]

In Ethiopia, there is a scarcity of published data on the spectrum of etiologic agents and risk factors of bacterial keratitis. Thus, this study was conducted to address these issues as well as to assess the *in-vitro* antimicrobial susceptibility of these bacterial isolates at Jimma University Specialized Hospital, Southwest Ethiopia.

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MATERIALS AND METHODS

A prospective study was conducted after securing ethical approval from the College of Public Health and Medical Sciences Research Ethical Review Committee and the Department of ophthalmology. Patients were informed about the purpose of the study, written informed consent was obtained from the study participants, and any information related to the patient and their clinical history was kept confidential. Culture results were sent to the responsible ophthalmologist so that the participants would benefit from the study.

This study included 24 corneal scrapings for bacteriological evaluation from patients clinically diagnosed with bacterial keratitis at Jimma University Specialized Hospital (JUSH) in Oromia, Southwest Ethiopia, between January 2012 and June 2012. Patients clinically diagnosed with bacterial keratitis who were willing to give informed consent were included in the study; whereas 4 patients with non-bacterial causes of Keratitis (3 fungal and 1 acanthamoebic), 5 patients with recent ocular surgery and 2 patients who took antimicrobial therapy within seven days before recruitment were excluded from the study. Patients with post-operative corneal infections were excluded for ethical reasons. The history and examination were focused on the following risk factors: corneal traumas, contact lens wear, herpetic keratitis, ocular surface diseases, lagophthalmos, use of steroid eye drops, presence of corneal foreign bodies or sutures, use of contaminated medications, blepharitis as well as history of systemic diseases particularly diabetes mellitus.

All the patients were examined on the slit-lamp biomicroscope and infectious diseases included in this study were diagnosed clinically by a group of ophthalmologists. After detailed ocular examinations using standard techniques [11], corneal scrapings were collected by ophthalmologists with a 20 gauge sterile needle. A topical anesthetic (0.5% tetracaine) was used when taking the corneal specimens. Two corneal scrapings were obtained from each case with separate needle by scraping the leading edge and base of the ulcer using short, firm strokes. One scraping was subjected to Gram stain. The second scraping was inserted into a 2 ml brain heart infusion broth (Oxoid, Hampshire, UK) tube. Then, it was immediately inoculated into chocolate and blood

agar. A chocolate and a blood agar plate were placed into a candle jar for fastidious bacterial pathogens which require CO₂, while one blood agar plate was placed into a cold chain to maintain the viability of the bacterial pathogens during transport. Three culture plates and one tube were incubated for each case with appropriate conditions.

After appropriate incubation, subcultures were made onto sheep blood agar (5%), chocolate agar, manitol salt agar and MacConkey agar (Oxoid, Hampshire, UK) using the standard methods. The inoculated media plates were incubated at their respective optimal temperatures; for example, blood agar and chocolate agar plates were incubated at 37°C with 5-10% CO₂. All other media were incubated at 37°C in aerobic conditions. The plates were examined after 24 and 48 hours. Growth, if any, was identified by standard methods.[12] A culture was considered positive when there was growth of the same organism on two or more media, confluent growth of a known ocular pathogen at the site of inoculation on one solid medium, growth in one medium with consistent direct microscopy findings, or growth of the same organism on repeated corneal scraping. [4]

The bacterial isolates from the corneal scrapings were identified up to species level based on different criteria which include morpho-cultural and biochemical characteristics [12]. The Optochin sensitivity test was also performed to identify *Streptococcus pneumoniae*. [13]

In vitro antibiotic susceptibility testing of the bacterial isolates was performed by Kirby-Bauer disc diffusion method. [12] Results were interpreted according to the Clinical Laboratory Standards Institute (CLSI) methodology as susceptible, intermediate and resistant. Data obtained was subjected to statistical analysis using SPSS for windows version 16.

RESULTS

Patients were classified according to their age into three groups: children (<10 years), 4.2%, adolescents (10-20 years), 8.3% and adults (> 20 years), 87.5%. Out of the 24 patients, 21(87.5%) were males with male: female ratio of 7:1. Of the 24 corneal scrapings subjected to culture, 20 (83.3%) had bacterial growth. The predominant isolate was *P. aeruginosa* (41.7%) and among those with positive gram stains, *S. aureus* (20.8%) was the predominant isolate. Causative organisms stratified by predisposing factors are summarized in Table 1.

Table 1: Risk factors and bacterial culture results of bacterial keratitis at Jimma University Specialized Hospital

Corneal culture result	Risk factors of Bacterial Keratitis				Total
	corneal trauma	Blepharitis	Herpetic keratitis (Herpes simplex)	Use of Contaminated medications	
	n (%)	n (%)	n (%)	n (%)	n (%)
<i>P. aeruginosa</i>	4 (44.4)	3 (42.9)	1(25)*	2 (50)	10 (41.7)
<i>S. aureus</i>	1 (11.1)	2 (28.6)	2 (50)	0	5 (20.8)
<i>S. marcescens</i>	2 (22.2)	0	0	1 (25)	3 (12.5)
<i>S. pneumoniae</i>	0	1 (14.3)	1(25)	0	2 (8.3)
No growth	2 (22.2)	1 (14.3)	0	1 (25)	4 (16.7)
Total	9 (100)	7 (100)	4 (100)	4 (100)	24 (100)

*Patient had multiple risk factors (Herpes simplex keratitis, Use of Contaminated medications and lagophthalmus).

At initial presentation, among those with bacterial keratitis, the majority (41.7%) had visual acuity of 6/24-6/60. At the end of the treatment, 7 patients (29.2%) had attained vision of 6/6 to 6/18, 12 patients (50.0%) had visual acuity of 6/24 to 6/60. Three patients had visual acuity detecting hand

movement and another patient with light perception. Nineteen (79.2%) of our patients had healed ulcers with treatment with no significant visual disability. One patient had progressed to endophthalmitis and was subjected to inadvertent evisceration.

Table 2: Visual acuity of bacterial keratitis patients (N= 24) at initial presentation (admission) and discharge

Visual acuity	On admission	On discharge
	n (%)	n (%)
6/6-6/18*	5(20.8)	7(29.2)
6/24-6/60*	10(41.7)	12(50.0)
Counting fingers	0	0
Hand movement	6(25.0)	3(12.5)
Perceived light	3(12.5)	1
Did not perceive light	0	1

* 6/6- 6/18= Normal vision, 6/24- 6/60= Moderate visual loss

The antimicrobial susceptibility pattern of bacteria was performed against eleven antimicrobial agents. [Table 3] More than half of Gram-negative bacteria showed resistance against trimethoprim-sulphamethoxazole (8;61.5%) and tetracycline (7; 53.8%); however, they were relatively highly susceptible to ciprofloxacin (11; 84.6%), amikacin (11; 84.6%), gentamicin (11; 84.6%), ceftriaxone (8; 61.5%), doxycycline (7; 53.8%), and chloramphenicol (7; 53.8%). *P. aeruginosa*, comprising the majority (10 isolates,76.9%) of the Gram-negative bacteria, were highly susceptible towards gentamicin (9, 90.0%), amikacin (8, 80.0%), ciprofloxacin (8, 80.0%), and ceftriaxone (7, 70.0%).

However, they showed a high resistance towards trimethoprim-sulphamethoxazole (7, 70.0%), tetracycline (5, 50.0%), and doxycycline (5,50.0%). *S. aureus* comprised 71.4% of the Gram-positive bacteria and showed a high level of susceptibility to ciprofloxacin (4isolates; 80.0%), amikacin (4,80.0%), gentamicin (4, 80.0%), vancomycin (4, 80.0%), doxycycline (4, 80.0%), and ceftriaxone (4, 80.0%). However, the entire *S. aureus* isolates were highly resistant to penicillin.

Table 3: Antimicrobial susceptibility pattern of bacterial isolates microbial keratitis at Jimma University Specialized hospital, Southwest Ethiopia.

Antibiotic	<i>P. aeruginosa</i> (n=10)		<i>S. aureus</i> (n=5)		<i>S. marcescens</i> (n=3)		<i>S. pneumoniae</i> (n=2)	
	S	R	S	R	S	R	S	R
penicillin	NT	NT	0	5 (100)	NT	NT	2 (100)	0
amikacin	9 (90)	1 (10)	5 (100)	0	3 (100)	0	0	2 (100)
chloroamphenicol	7 (70)	3 (30)	3 (60)	2 (40)	2 (66.7)	1 (33.3)	2 (100)	0
gentamicin	10 (100)	0	5 (100)	0	2 (66.7)	1 (33.3)	0	2 (100)
vancomycin	NT	NT	5 (100)	0	NT	NT	2 (100)	0
erythromycin	NT	NT	4 (80)	1(20)	NT	NT	2 (100)	0
ciprofloxacin	10 (100)	0	5 (100)	0	3 (100)	0	2 (100)	0
trimethoprim-sulphamethoxazole	3 (30)	7 (70)	4 (80)	1(20)	2 (66.7)	1 (33.3)	1 (50)	1 (50)
tetracycline	5 (50)	5 (50)	3 (60)	2 (40)	1(33.3)	2 (66.7)	1 (50)	1(50)
doxycycline	5 (50)	5 (50)	4 (80)	1 (20)	3 (100)	0	2 (50)	0
ceftriaxone	9 (90)	1 (10)	4 (80)	1 (20)	1 (33.3)	2 (66.7)	1 (50)	1(50)

NT= not tested, S= susceptible, R= resistance

DISCUSSION

Bacterial keratitis is an ophthalmic emergency requiring immediate institution of treatment. In the absence of laboratory diagnosis the initial therapy is usually broad spectrum intensive antibiotic treatment. Specific therapy should be based on laboratory data and directed towards the causative agents and antibacterial susceptibility results. [2]

We found that corneal trauma was the most common predisposing factor in our patients. This is consistent with some studies [2, 14], but not others which reported contact lens wear [15, 16] This difference may be attributed to low prevalence of contact lens usage in Ethiopia, less than 1%.

A total of 24 corneal scrapings were obtained from patients clinically diagnosed with microbial keratitis for microbiological evaluation during the study period. Of the 24 corneal scrapings subjected to culture, 20 (83.3%) had bacterial growth. This is higher than other similar studies around the world [14, 15]. This may be due to bias in selection of the study subjects. In positive cultures, *P. aeruginosa* (41.7%) was the most common pathogen, which is similar to the results of several other studies [2, 17, 18], followed by *S. aureus* (20.8%). *Pseudomonas* grows better in the cornea than in any known culture media and causes infection when mechanical trauma of the corneal epithelium occurs. It produces exotoxin A, which causes tissue necrosis leading to corneal ulceration [4]. However, other similar studies [19, 20] reported *S. pneumoniae* as the most common isolate in bacterial keratitis. One study in India [1] reported *P. aeruginosa* and *S. pneumoniae* as a predominant isolates of bacterial keratitis with equal frequency. Other studies [14, 21] reported *Staphylococcus* spp. as a predominant isolates. This may be due to the variation with the patient population, health of the cornea, geographic location and climate, and also tends to vary somewhat over time. [19]

In general, the ocular isolates identified in this study were similar to those of many other studies conducted in different areas either nationally or internationally with few exceptions. Even though the main bacteria known to cause severe keratitis are *P. aeruginosa* and *S. aureus*, the prevalence and degree of occurrence of corneal pathogens over others are dependent on the geographic location and on the lo-

cal population. Resistance and sensitivity based on *in vitro* testing may not reflect true clinical response to an antibiotic because of the host factors and penetration of the drug. [5] However, these results do provide information that allows a clinician to make rational choice of an initial regimen for treatment of ocular pathogens. [22]

Based on results from susceptibility testing in this study, most Gram-negative bacilli (84.6%) were susceptible to amikacin, gentamicin, and ciprofloxacin; whereas most Gram-positive organisms (85.7%) were susceptible to vancomycin, ciprofloxacin and doxycycline. The sensitivity of gentamicin against Gram-negative bacilli was 84.6% (11 of 13 isolates) and the coverage of this antibiotic for *P. aeruginosa* was 90.0% (9 of 10 isolates). This is comparable with similar studies done in Nigeria, [4] and Iran [23]. However, a study conducted in India [24] reported low sensitivity of gentamicin against *P. aeruginosa*. Amikacin had high coverage against *S. aureus* (80.0%, 4 of 5). This is consistent with similar studies conducted in India [5, 22, 24]; the emergence of bacterial resistance in these studies was due to characteristics of the pathogens, antibiotic-prescribing practices including the widespread use of systemic antibiotics, and health care guidelines. Other contributing factors may include improper dosage regimens, misuse of antibiotics for viral and other non-bacterial infections, extended duration of therapy as well as globalization and migration. [6]

The susceptibility of vancomycin against *S. aureus* was 80.0% (4 of 5 isolates). This finding is in agreement with a study conducted in India [22]. Gentamicin covered against 4 (30.8%) of the 7 Gram-positive cocci isolates and had high coverage against *S. aureus* (90.0%, 9 of 10). This result is consistent with similar studies conducted in India [4, 5, 22], and Nigeria [10]. *P. aeruginosa*, were highly sensitive towards amikacin (8, 80.0%), ciprofloxacin (8, 80.0%), and ceftriaxone (7, 70.0%). Similar results have been reported for ciprofloxacin from similar studies in Saudi Arabia [32] and Nigeria. [33] However, study in India [24] reported low coverage of ciprofloxacin for *P. aeruginosa*.

In Ethiopia, it is in common practice that antibiotics can be purchased without prescription, which leads to misuse of antibiotics. This may contribute to the emergence and spread of antimicrobial resistance. Other factors may include availability of suboptimal quality or substandard antimicrobial drugs, over usage of a particular antimicrobial agent, poor sanitation, contaminated food and cross-contamination

from humans or animals [4, 13]. As a result the susceptibility patterns of bacteria to various antimicrobial agents may vary from place to place and in the same place from time to time [7].

Conclusion: In conclusion, corneal trauma was the most common risk factor for bacterial keratitis followed by blepharitis. Bacteriological analysis of corneal scrapings showed that *P. aeruginosa* was the most common isolate followed by *S. aureus*; the antibiotic with the greatest coverage was ciprofloxacin. As drug resistance among bacterial pathogens is an evolving process, routine surveillance and monitoring studies should be conducted to provide update and most effective empirical treatment for bacterial keratitis.

In addition, the present study enrolled limited numbers of study subjects; more studies will be needed to more firmly establish area specific microbial prevalences and risk factors.

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