



The Bacterial Profile and Microbial Susceptibility of Acute and Chronic Dacryocystitis in Matini Hospital, Kashan, Iran

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Abstract

Background: Recent studies have shown an increasing incidence of antibiotic resistance in dacryocystitis. Management of diseases may include determining microbial agents and choosing appropriate antibiotics for treatment.

Objectives: This study aimed to present the best treatments for dacryocystitis. To this end, specimens' microbiology and antibiotic susceptibility were examined in patients with dacryocystitis in the microbiology laboratory of the Kashan University of Medical Sciences.

Methods: This cross-sectional study was performed on 172 patients presenting with acute and chronic dacryocystitis at the Matini Hospital, Kashan, between 2017 - 2018. Patient characteristics, culture isolates, and antimicrobial susceptibility data were collected. The PCR assay of the *mecA* gene was performed in all methicillin-resistant *Staphylococcus* isolates.

Results: The most common bacteria were coagulase-negative staphylococci (CoNS), *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. The majority of the isolated microbes were sensitive to rifampicin, linezolid, amikacin, and gentamicin. In Gram-negative bacilli, nine of the isolates were extended-spectrum beta-lactamase positive. The PCR test showed the frequency of *mecA* gene of resistant *S. aureus* and resistant CoNS isolates to be 40 and 46.3%, respectively.

Conclusions: Coagulase-negative staphylococci were the most frequently isolated bacteria. The highest antibiotic susceptibility was observed to rifampin, linezolid, amikacin, and gentamicin. A high percentage of CoNS carried the *mecA* gene.

Keywords: Drug Resistance, Methicillin Resistance, Dacryocystitis, Antibiotic Susceptibility

1. Background

Dacryocystitis is an infection of the nasolacrimal sac caused by nasolacrimal duct obstruction. Obstruction of the nasolacrimal duct leads to mucous secretion in the lacrimal sac. In this situation, microorganisms, such as normal flora or pathogens, can be colonized within the lacrimal sac (1). Clinical symptoms of dacryocystitis include pain, redness, and swelling over the inner side of the lower eyelid (2). The microbial agents of this disease have not been described in different geographical areas. *Staphylococcus* spp., *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* are the most common causes of dacryocystitis (1). Studies reported that *Staphylococcus aureus*, coagulase-negative staphylococci (CoNS), *Streptococcus* spp., and Gram-negative bacilli were the most frequently isolated bacteria (3). Moreover, antibacterial susceptibility tests showed that Gram-positive and Gram-

negative bacteria were most sensitive to rifampicin, gentamicin, and amikacin (3, 4).

2. Objectives

This study was performed to determine bacterial pathogen agents of dacryocystitis and its antimicrobial susceptibility patterns in patients. To this end, we examined the microbiology and antibiotic susceptibility of ocular samples to provide the best treatment for dacryocystitis.

3. Methods

3.1. Study Design

This cross-sectional study was performed from June 2017 to June 2018 on patients with dacryocystitis admitted to the ophthalmology clinic of the Matini Hospital,

Kashan, Iran. An ophthalmologist examined all the patients to see whether they had clinical signs of duct obstruction. The patients completed a demographic questionnaire on age, sex, place of residence, and occupation. Furthermore, information concerning the type of disease, the form of discharge, clinical signs, and background illness was obtained from the patients.

3.2. Bacterial Isolation

Swabs taken from the patients were collected in the sterile tryptic soy broth medium (Merck, Germany). The specimens were transported to the microbiology laboratory of the Kashan University of Medical Sciences immediately. One of the swabs was used for Gram staining, and the other was inoculated on a blood agar with 5% sheep blood, MacConkey agar, and chocolate agar (Merck, Germany). The media were inoculated under different conditions: (1) at 37°C for 24 h in aerobic conditions; (2) at 37°C in the presence of 10% CO₂ in a candle jar for 48 h; and (3) at 37°C in an anaerobic jar loaded with Gaspak for 48 h.

The isolates were identified based on microscopic morphology, staining characteristics, and cultural and biochemical properties using oxidase, catalase, coagulase, DNase, optochin, CAMP, IMViC, and susceptibility to bile tests, growth at mannitol salt agar and 6.5% NaCl (Merck, Germany). The antibiotic susceptibility test was performed using the Kirby-Bauer method according to the Clinical and Laboratory Standards Institute (CLSI) protocol (1). Finally, the plate was incubated at 37°C for 24 h. The zone of inhibition around the disk was measured and compared to CLSI 2018 charts (5). The antibiotics used for antimicrobial susceptibility are shown in Table 1 (Mast, UK).

3.3. Detection of Methicillin-Resistant *Staphylococcus*

In the current study, methicillin-resistant *Staphylococcus* (MRS) was detected using a cefoxitin disk. In interpreting the results, each growth inhibition zone < 21 mm (for *S. aureus*) and < 24 mm (for CoNS) was considered a methicillin-resistant strain (6).

3.4. Detection of Extended-Spectrum Beta-Lactamase

The combination of ceftazidime disks alone and ceftazidime (30 µg)/clavulanic acid (10 µg) was placed at a space of 2 cm on Mueller-Hinton agar (Merck, Germany) was used for *Enterobacteriaceae* and non-fermented Gram-negative bacilli. Extended-spectrum beta-lactamase (ESBL) production was distinguished with an increased width about the disk of ceftazidime/clavulanic acid by 5 mm or more (7).

3.5. Molecular Identification Methicillin-Resistant *Staphylococcus*

3.5.1. DNA Extraction

The boiling method was used to extract DNA. For this purpose, bacteria isolates were cultured on the blood agar (Merck, Germany). Then, two loops filled with pure colonies were added to the sterile microtube containing 500 µL deionized DNase and RNase-free water and placed in boiling water for 15 min. Afterward, the samples were placed on ice for 2 - 3 min and centrifuged at 10,000 g for 7 min. The supernatant containing DNA was stored at -20°C. An amount of 4 µL of this supernatant was applied as the template for PCR reaction.

3.5.2. PCR Reaction

Methicillin-resistant *Staphylococcus* isolates were detected by recognition of the *mecA* gene using the PCR technique. The characteristics of the used primers are shown in Table 2. The amplification condition for the *mecA* gene was an initial denaturation step at 94°C for 4 min, 35 cycles of denaturation at 94°C for 45 s, annealing at 50°C for 45 s, extension at 72°C for 1 min, and a final extension at 72°C for 2 min. Finally, the PCR products were run in 1% agarose gel. Also, *S. aureus* ATCC 33591 and *S. epidermidis* ATCC 12228 were used as the positive and negative controls, respectively.

3.6. Statistical Analysis

The frequency percentage of all the data was calculated. Chi-square distribution was used to test the qualitative distribution. A P-value < 0.05 was considered significant.

4. Results

A total of 172 eye specimens were analyzed. Of the patients, 88.4% lived in urban areas, and 11.6% lived in rural areas. Moreover, 56.2 and 43.8% of the patients were male and female, respectively. Also, 82.9% of the patients were under 30 years of age, and 17.1% of them were between 30 - 61 years of age, with the minimum age being 6 days and the maximum age being 96 years. According to the symptoms, 30.3% of the patients had chronic dacryocystitis, and 69.7% of them had acute dacryocystitis. Further, 41.8% of the patients had purulent discharge, and 58.2% of them had mucous discharge. Swelling and redness were observed in 5.4% of the patients (100%). In this study, culture was positive in 96.9% of the specimens, and more than one bacterium strain were isolated from some of the specimens. Frequency of Gram-positive and Gram-negative bacterial agents isolated from dacryocystitis patients show in Figures 1 and 2.

The antibiotic susceptibility results are shown in Tables 3 - 5. Ceftazidime resistance was observed in

Table 1. The Antibiotics (Mast, UK) Used for Antimicrobial Susceptibility Test

Gram-Positive Bacteria	Gram-Negative Bacteria	Non-fermented Gram-Negative Bacteria
PG: penicillin (10 µg)	AK: amikacin (30 µg)	AK: amikacin (30 µg)
Fox: cefoxitin (30 µg)	GM: gentamicin (10 µg)	GM: gentamicin (10 µg)
GM: gentamicin (10 µg)	TS: cotrimoxazole (25 µg)	CAZ: ceftazidime (30 µg)
E: erythromycin (15 µg)	CIP: ciprofloxacin (5 µg)	MEM: meropenem (10 µg)
T: tetracycline (30 µg)	CTX: cefotaxime (30 µg)	TS: cotrimoxazole (25 µg)
TS: cotrimoxazole (25 µg)	PTZ: piperacilin-tazobactam (110 µg)	ATM: aztreonam (30 µg)
RP: rifampicin (5 µg)	AUG: augmentin (30 µg)	CIP: ciprofloxacin (5 µg)
CIP: ciprofloxacin (5 µg)	AP: ampicillin (10 µg)	CTX: cefotaxime (30 µg)
LZD: linezolid (30 µg)	CAZ: ceftazidime (30 µg)	

Table 2. Sequences of Primers and PCR Product Size of *mecA* Gene

Primer	Primer Sequence	Amplicons Size (bp)	Ref.
<i>mecA</i> F	5'-ACTGCTATCCACCCTCAAAC-3'	160	(8)
<i>mecA</i> R	5'-CTGGTGAAGTTGTAATCTGG-3'		

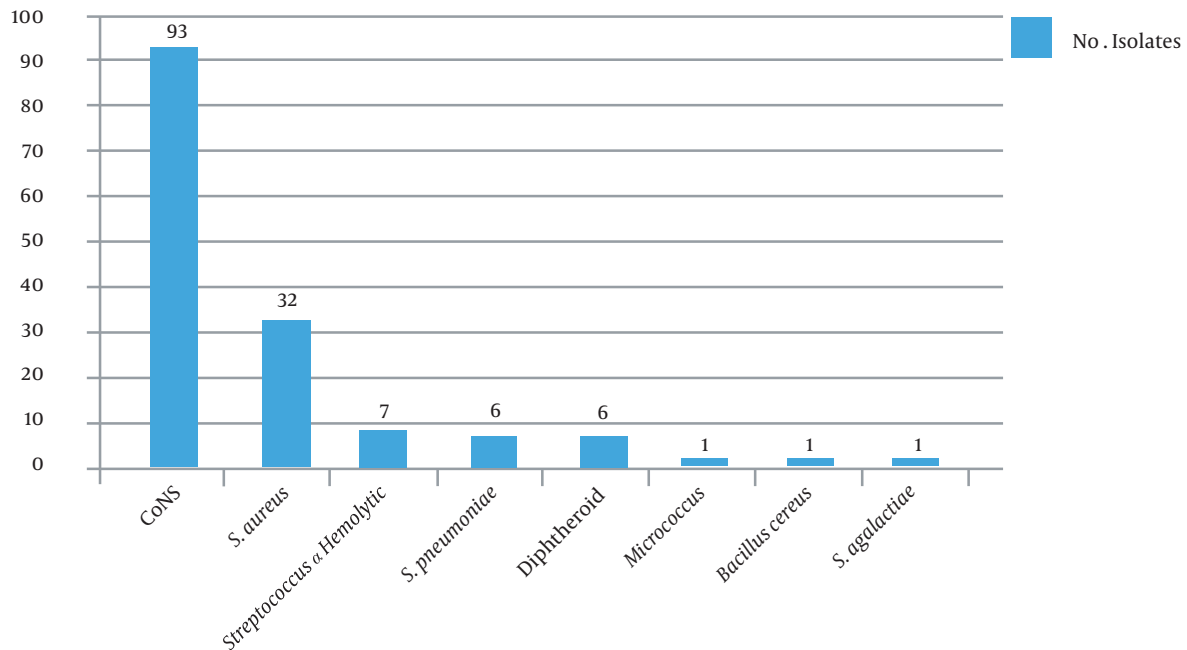


Figure 1. Frequency of Gram-positive bacterial agents isolated from dacryocystitis patients

ten isolates of *Enterobacteriaceae* and nine isolates of non-fermented Gram-negative bacilli. Using the combination disk test method, we detected ESBL activity in seven isolates (70%) of *Enterobacteriaceae*. The frequency of ESBL in non-fermented Gram-negative bacteria was two isolates (22.2%). Extended-spectrum beta-lactamase-producing bacteria were *Klebsiella* (n = 2), *Yersinia* (n = 2), *Escherichia coli* (n = 1), *Citrobacter* (n = 1), *Enterobacteriaceae* (n = 1), *P. aeruginosa* (n = 1), and *Stenotrophomonas*

(n = 1). Of the 32 *S. aureus* isolated in this study, ten were confirmed as methicillin-resistant *S. aureus* (MRSA). Moreover, 66 of the 93 CoNS isolated were verified as methicillin-resistant CoNS (MR-CoNS) using the phenotyping method. Methicillin-resistant *Staphylococcus* isolates were recognized via identifying the *mecA* gene using the PCR method. The results showed that 40% (n = 4) of the MRSA isolates and 89.4% (n = 59) of the MR-CoNS isolates carried the *mecA* gene, respectively.

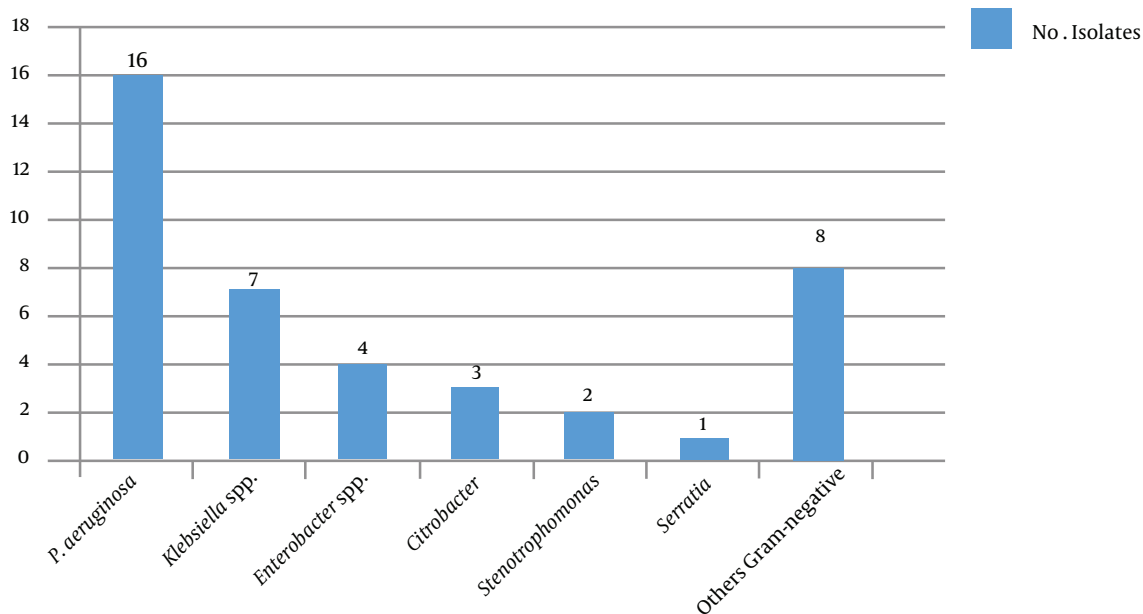


Figure 2. Frequency of Gram-negative bacterial agents isolated from dacryocystitis patients

Table 3. Antibiotic Sensitivity Pattern of Gram-Positive Bacteria by Disk Diffusion Method^a

Organism/Isolate	PG	FOX	GM	E	T	TS	RP	CIP	LZD
CoNS/93	13 (14)	57 (61.3)	84 (90.3)	32 (34.4)	60 (64.5)	74 (79.6)	88 (94.6)	76 (81.7)	91 (97.8)
<i>Staphylococcus aureus</i> /32	3 (9.4)	22 (68.7)	32 (100)	15 (46.8)	26 (81.25)	26 (81.25)	28 (87.5)	30 (93.75)	32 (100)
<i>S. α hemolytic</i> /7	-	-	-	3 (42.8)	4 (57.1)	-	7 (100)	-	5 (71.4)
<i>S. β hemolytic</i> /1	-	-	-	1 (100)	-	-	1 (100)	-	1 (100)

^a Values are expressed as No. (%).

Table 4. Antibiotic Sensitivity Pattern of Non-fermented Gram-Negative Bacteria by Disk Diffusion Method^a

Organisms/Isolate	AK	GM	CAZ	MEM	TS)	ATM	CIP)	CTX
<i>Pseudomonas aeruginosa</i> /16	14 (87.5)	13 (81.2)	8 (50)	12 (75)	-	2 (12.5)	12 (75)	-
<i>Acinetobacter baumannii</i> /7	5 (71.4)	4 (57.1)	1 (14.2)	5 (71.4)	-	-	-	4 (57.1)
<i>Burkholderia</i> /1	-	-	1 (100)	1 (100)	1 (100)	-	-	-

^a Values are expressed as No. (%).

Table 5. Antibiotic Sensitivity Pattern of *Enterobacteriaceae* Bacteria by Disk Diffusion Method^a

Organisms/Isolate	AK	GM	TS	CIP	CTX	PTZ	AUG	AP	CAZ
<i>Enterobacterspp.</i> /3	2 (66.6)	2 (66.6)	1 (33.3)	2 (66.6)	2 (66.6)	2 (66.6)	2 (66.6)	2 (66.6)	-
<i>Klebsiellaspp.</i> /4	4 (100)	4 (100)	3 (75)	4 (100)	4 (100)	4 (100)	4 (100)	2 (50)	1 (25)
<i>Escherichia coli</i> /2	2 (100)	2 (100)	1 (50)	2 (100)	2 (100)	1 (50)	1 (50)	1 (50)	1 (25)
<i>Yersinia enterocolitica</i> /4	3 (75)	4 (100)	4 (100)	4 (100)	3 (75)	3 (75)	1 (25)	-	2 (50)
<i>Citrobacter</i> /2	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	-	-	1 (25)

^a Values are expressed as No. (%).

5. Discussion

Dacryocystitis is a common lacrimal duct infection. The lacrimal system directs tears from the lacrimal glands to the nasal cavity (9). Obstruction of the lacrimal duct causes tear retention and, consequently, bacterial accumulation in this area (10). In this study, 96.9% of the specimens had positive culture, and 12 species of bacteria were isolated. Our results demonstrated that CoNS and *S. aureus* were the most frequent Gram-positive bacteria, and *P. aeruginosa* was the most Gram-negative isolated organism in the patients. The results of several other studies confirm this finding (11, 12).

Women are more commonly affected by lacrimal duct obstruction because of the lower nasolacrimal duct and hormonal factors (13). In our study, the number of female patients was higher than male ones in the age groups 1 - 30 years and > 60 years. The reason may be prolonged presence in the kitchen, hot air, the use of different types of cosmetics, and a weakened immune system in old age. The present study results also showed that the disease was predominantly acute in the age groups < 1 year and 1 - 30 years and chronic in the age groups between 31 - 60 years and > 60 years (Table 6). Previous studies also showed that patients < 30 years had significantly acute dacryocystitis (4, 14). Identification of bacterial agents that cause dacryocystitis and their antibiotic sensitivity is essential in treating and preventing this complication. According to studies performed on the microbial profile of dacryocystitis, there has been considerable variation in microbial agents and drug management (11). However, during the past 50 years, the prevalence of microbial agents of dacryocystitis has changed. In the 1930s, *S. pneumoniae* was the most common isolated agent of this infection. However, today, it is much less frequent than *S. epidermidis* (1).

Nowadays, MRSA is frequently detected in ocular infections worldwide, and it has been determined that methicillin-resistant *S. epidermidis* can cause ophthalmic infections and blindness (15). In our study, the predominant organism in the acute and chronic forms of the disease was CoNS. In the age groups 1-30 years and > 60 years, in addition to CoNS, MRSA was also the cause of the disease (Table 6). In other studies, Gram-positive organisms were documented in 78.58% of organisms (16). Chaudhary reported that *S. epidermidis* (89.62%) was the most common isolate from lacrimal sac infection, followed by *S. aureus* (17).

In the current study, different antibiotics were used to study the microbial susceptibility of bacterial strains. In the group of Gram-positive bacteria, the most common isolated organism, CoNS, had sensitivity to linezolid (97.8%), rifampicin (94.6%), gentamicin (90.3%), and ciprofloxacin (81.7%). Also, *S. aureus* showed the highest susceptibility to linezolid (100%), gentamicin (100%), and ciprofloxacin (93.75%). Among all the analyzed *P. aeruginosa* isolates, the

highest resistance was observed to aztreonam. The results of Negm et al.'s study confirm our results, showing that Gram-negative isolates were more sensitive to the third generation of cephalosporins, such as cefotaxime (80%) and ceftazidime (60%) (2). In the *Enterobacteriaceae* group, the highest (100%) sensitivity was to amikacin, gentamicin, ciprofloxacin, cefotaxime, piperacillin-tazobactam, and augmentin in *Klebsiella*. Thus, these drugs can be used to treat dacryocystitis caused by *Klebsiella* strains. However, sensitivity to cefotaxime, ciprofloxacin, amikacin, and gentamicin was the highest (100%) in the *E. coli* group (Tables 3 - 5). Sun et al. showed that among aminoglycosides, amikacin had the highest effectiveness toward Gram-negative isolates (18), which agrees with our results. Also, the highest resistance in this group was to ampicillin and ceftazidime in *Klebsiella* and augmentin (amoxicillin/clavulanic acid) and cotrimoxazole in *E. coli*. Amin et al. also reported resistance to ampicillin and augmentin in *Enterobacteriaceae* (19).

Although the prevalence of MRSA infection was determined based on cefoxitin's resistance pattern, the gold standard for identifying MRSA is to detect the *mecA* gene (20). Getahun et al. and Nithya et al. showed a significant variation in the prevalence of MRSA ocular infections geographically at different times (21, 22). In this study, four MRSA strains and 59 MR-CoNS strains had the *mecA* gene. Of the 125 *Staphylococcus* isolates, 63 (50.4%) were carried. In other studies, the prevalence rate of MRSA was 32% among *S. aureus* isolates of dacryocystitis (23). Finally, the microbial profile may vary widely among geographic regions and during different periods (6). The prevalence of Gram-negative bacteria, including *P. aeruginosa* and *Acinetobacter baumannii*, and resistance to antibiotics were higher in this study than in other studies (10, 11).

5.1. Conclusions

CoNS were the most frequently isolated bacteria. The highest antibiotic susceptibility was observed to rifampin, linezolid, amikacin, and gentamicin. A high percentage of CoNS carried the *mecA* gene.

Footnotes

Authors' Contribution: Study concept and design, A. NA., R. M., and F. B.; Analysis and interpretation of the data, F. B. and M. SH; Drafting of the manuscript, F. B. and A. NA; Critical revision of the manuscript for important intellectual content, F. M. and A. NA; Statistical analysis, H. A.

Conflict of Interests: All the authors declare that there are no conflicts of interest.

Ethical Approval: The work was performed by the principles of the Declaration of Helsinki, and ethical approval was gained (IR.KAUMS.REC.1396.30).

Table 6. Distribution of Patients According to Age, Gender, Form of Disease, and Organism^a

Age (y)	Form of Disease		Gender				Common Organism	
	Acute	Chronic	Acute		Chronic		Acute	Chronic
			M	F	M	F		
< 1	103 (86)	17 (14)	52 (50.4)	51 (49.5)	10 (58.8)	7 (41.2)	CoNS	CoNS
1-30	20 (69)	9 (31)	6 (30)	14 (70)	4 (44.4)	5 (55.5)	CoNS	CoNS, <i>S. aureus</i>
31-60	2 (13.3)	13 (86.6)	2 (100)	0	7 (53.8)	6 (46.2)	CoNS	CoNS
> 60	5 (31.25)	11 (68.75)	1 (20)	4 (80)	3 (27.2)	8 (72.7)	CoNS	<i>S. aureus</i>

^a Values are expressed as No. (%) unless otherwise indicated.

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