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Original research

# Identification of bacterial isolates from nasolacrimal duct infection in children with congenital nasolacrimal duct obstruction from Feiz teaching hospital, Isfahan

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## Abstract

Dacryocystitis is a lacrimal sac and duct inflammation. It can be inherited or congenital. Two primary forms are acquired dacryocystitis, acute and chronic. The goal of this study is to recognize common bacteria causing nasolacrimal duct infection in children with congenital nasolacrimal duct obstruction and to determine their antimicrobial susceptibility profiles. This cross-sectional research was conducted from January to February 2017 in the Department of Ophthalmology affiliated to Isfahan University of Medical Sciences (center of Iran). Using phenotypic and genotypic approaches, identification of specimens was performed. Disc diffusion method was used for checking antibiotic susceptibility. All of the 59 isolates from the culture of specimens belonged to Gram-positive cocci. *Staphylococcus epidermidis* was the predominant species (n= 44, 74.6%) followed by *Staphylococcus aureus* (n= 11, 18.6%), *Staphylococcus haemolyticus* (n= 2, 3.4%) and each of *Staphylococcus saprophyticus*, and *Streptococcus pneumoniae* (n= 1, 1.7%). Totally, the highest resistance was found against erythromycin and tetracycline while, chloramphenicol, and ciprofloxacin showed the highest susceptibility. The current research is useful in evaluating the suitable antibiotic in our area for the systemic treatment of dacryocystitis. The most effective agents against the most common isolates were chloramphenicol and ciprofloxacin. Since the bacteriology of nasolacrimal duct infections varies from region to region, it is recommended that further studies in other areas of our country can be detected the etiology of bacterial pathogens involved in acute infections.

**Keywords:** Dacryocystitis, Antibiotic resistance, Congenital nasolacrimal duct obstruction, Bacteriology

## 1. Introduction

Dacryocystitis is an inflammation of the lacrimal sac, usually followed by a blockage of the nasolacrimal duct [1]**.** Dacryocystitis can be seen both in acute and chronic forms. Acute type of dacryocystitis is an acute inflammation of the lacrimal sac, the most important clinical symptoms of which include discomfort, redness and swelling, and can be seen in 23 percent of cases of lacrimal abscess [2, 3]. The chronic form of dacryocystitis is more common than the acute form and is frequently associated with conjunctivitis [4].

Approximately 60-90% of all cases of lacrimal sac infection are related to bacterial dacryocystitis [5]. The microbial spectrum of the dacryocyst depends on its acute or chronic form. In most cases, Gram-positive bacteria are separated from acute dacryocystitis, while in the chronic form, Gram-negative bacteria are predominant. The most important species isolated

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from children include *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*, while *Staphylococcus epidermidis*, *S. aureus*, *S. pneumoniae*, and *Pseudomonas aeruginosa* are the most causative agents in adults [6]. Oral antibiotics, anti-inflammatory drugs and local hot compresses were used in acute dacryocystitis therapy methods, whereas definitive treatment of chronic dacryocystitis is done with dacryocystorhinostomy [7].

The study of the status of drug resistance in dacryocystitis is important in two aspects: first, according to studies, approximately one-third of bacteria isolated are resistant to antibiotics, and on the other hand, because in most cases, the treatment of patients is empirically and without culture data if treatment failure results in consequences such as cellulitis, meningitis, abscess, and even life-threatening conditions. In addition, the pattern of drug resistance varies in different regions [8]. Early identification of microbial agents and awareness of the drug susceptibility pattern is important for effective treatment. The choice of antibiotic therapy for dacryocystitis usually depends on the age of the patient, the condition of the patient (acute or chronic), the type of microorganism and drug present, and the drug resistance pattern. Therefore, epidemiologic studies are necessary for identifying and managing cases of bacterial dacryocystitis [5, 6].

Since the range of bacteriology may differ by geographical region, and very few studies have been recorded from Central Iran on lacrimal sac bacteriology, the present study was aimed at identifying bacteria involved in acute dacryocystitis in a given population and investigating the trend of antibiotic susceptibility in the province of Isfahan.

## 2. Materials and Methods

2.1 *Study design, period, and area*

A cross-sectional study was conducted from January to February 2017 among dacryocystitis diagnosed patients attending at Ophthalmology Outpatient of Feiz teaching hospital, Center of Iran.

*2.2 Inclusion/exclusion criteria*

All children aged 6 years with a history of dacryocystitis were referred to the selected clinic of ophthalmology for sampling and 59 dacryocystitis cases were eligible for microbiological analysis. Also, children with severe lid irritation due to persistent discharge were included in this study. However, people over 6 years old, the patients with the above symptoms who had received either topical or systemic antibiotics for the past week and all cases of canalicular obstruction were excluded from the study.

*2.3 Sampling*

After aseptically cleaning the surrounding area, specimens for microbiological analysis were obtained by sterile dacron swabs from the lacrimal sac, by applying pressure over the lacrimal sac and allowing the purulent material to reflux through the lacrimal punctum. The specimens were collected with the help of an ophthalmologist and sent for microbiological analysis.

*2.4 Microbiological analysis and bacterial identification*

Specimens were inoculated on BHI broth, chocolate agar, and blood agar (Oxiod, Hampshire, UK). Then, the inoculated media were incubated at 35-37 $^{o}$C for 24 to 48 hours. In addition, in the presence of 5-10 % carbon dioxide, chocolate plates were incubated at 37 $^{o}$C for 24 to 48 hours. Identification of the isolates was performed using different biochemical as well as standard microbiological tests. To identify Gram-positive isolates Standard biochemical and microbiological tests were included in order (Catalase, Coagulase, PYR, optochin susceptibility, etc.). The oxidase, novobiocin, and bacitracin tests were used for the identification of coagulase-negative staphylococci. Novobiocin disc is used to differentiate *Staphylococcus saprophyticus* from other coagulase-negative staphylococci.

*2.5 Antibiotic susceptibility pattern*

Antibiotic susceptibility pattern was performed by the disc diffusion method based on the Clinical and Laboratory Standards Institute (CLSI) recommendation [9]. The following antibiotic disks were used; ampicillin (10 µg), chloramphenicol (30 µg), gentamicin (10 µg), tetracycline (30 µg), amoxicillin (20 µg), ciprofloxacin (30 µg), ceftriaxone (30 µg), erythromycin (15 µg). Also, *Escherichia coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as control strains.

*2.6 DNA extraction and Polymerase chain reaction (PCR)*

The DNA template was prepared as per the method of Dilhari *et al.* with slight alteration [10]. 200 µl of phosphorus buffer saline (PBS) was taken in microcentrifuge tubes and a loopful of each isolate was mixed with the nuclease-free water thoroughly in each microcentrifuge tube. The suspended isolates in microcentrifuge tubes were then treated with boiling water for 15 minutes. After heat treatment, centrifuge tubes were centrifuged at 10,000 rpm for 10 minutes. 100 µl of the supernatant containing genomic DNA transfer in a new tube and it was used for subsequent PCR amplification. In our study, isolates were screened for the presence of the *16s rRNA*, *femA*, and *Se705* genes. The sequences of primers as shown in Table 1. The PCR reactions were conducted in a total volume of 25 µL containing the following: 12.5 µl of Master Mix (Ampliqon, Denmark), 11 µl distilled water, 1 µl template DNA and 0.5µl primers. PCR assay was performed in a DNA Thermal Cycler 480 (Applied Biosystems, USA). For amplification *femA*, and *Se705* genes, the PCR program was set at: denaturation for at 5 minute 94 °C, 29 cycles of 94 °C for 30 seconds, 53 °C for 30 seconds, 72 °C for 30 seconds and a final extension step of 72 °C for 5 minutes. Amplification of *16s rRNA* according to the following program: initial activation of 94 °C for 5 min, 35 cycles of 94 °C for1 min, 56 °C for1 min and 72 °C for 1 min and a final extension of 72 °C/7 min. Each amplification reaction included a negative control (no-DNA template control). Five microliters of the amplified DNA products were run on 1% agarose gel with 1 X TAE (Tris/Acetate/EDTA) buffer, stained with safe stain load dye (CinnaGen Co., Iran) and visualized under ultraviolet illumination.

*2.7 Data analysis*

The data were entered and analyzed using SPSS version 16 statistical software program. A p-value <0.05 was considered as a significant association between the variables which were tested.

## 3. Results

*3.1 Patients' demography*

In the present study, 59 clinically diagnosed patients of dacryocystitis in children and both sexes were studied over a period of two months. The average age of the patients was studied 3 years (range <1–6 years), with a males' predominance 57.6% (n=34) compared to female 42.4% (n=25) with male and female ratio 34:25. Although, a high frequency of dacryocystitis was reported in males than females, a significant difference between males and females (P= 0.36) was not observed. The most common age groups were the group under 1-2 years (33.9%) and the lowest age group was 5 years (5.1%).

*3.2 Bacterial isolates*

From 59 positive samples, five different species were isolated. As shown in Table 2, *S. epidermidis* was the most commonly isolated organism followed by *S. aureus*, then *S. hemolyticous*. *S. saprophyticus*, and *S. pneumoniae* only detected one isolate respectively.

*3.3 Antimicrobial susceptibility test*

The most effective antibiotics against all organisms were chloramphenicol, and ciprofloxacin, each showing affectivity of 100% (Table 3), followed by cefoxitin and gentamycin with 61% and 49.7% while the highest rate of resistance seen was to erythromycin, amikacin, and tetracycline, with at least one resistant organism present in 40 of 59 (67.8%) cultures and 30 of 59 (50.8%), and 30 of 59 (50.8%), respectively.

*3.4 Sequencing*

*Staphylococcus hominis* strain EFS 16S ribosomal RNA gene, partial sequence gene with accession number "MG786536.1" were added in the database.

## Discussion

This prospective study of 59 patients was done to determine the type of bacteria involved in patients with acute dacryocystitis. The spectrum and incidence of pathogens, as well as our study, were so obvious that we could isolate five different species of bacteria. Microbial ocular surface flora consists predominantly of Gram-positive microorganisms, namely staphylococci and diphteroids [12]. The occurrence and severity of dacryocystitis depend on various factors, such as the geography of the area and the type of microbial agent [10].

Badhu *et al.* (2006) revealed that in Nepal the most common microorganism was *S. pneumoniae*, while in some countries like Saudi Arabia, China,

Table 1. Sequences of primers used for the PCR

| Primer | Sequence (5'-3') | Amplicon size (bp) | Annealing (◦C) | References |
|---|---|---|---|---|
| 16S rRNA | AGAGTTTGATCCTGGCTCAG | 1500 | 57 | (30) |
| | TACGGYTACCTTGTTACGACTT | | 55 | |
| Se705 | ATCAAAAAGTTGGCGAACCTTTTCA | 125 | 60.6 | (31) |
| | CAAAAGAGCGTGGAGAAAAGTATCA | | 59.8 | |
| femA | CGATCCATATTTACCATATCA | 451 | 50 | (32) |
| | ATCACGCTCTTCGTTTAGTT | | 55 | |

Table 2. Frequency of isolated microorganisms in patients with dacryocystitis

| Isolate type | Frequency (%) (n=59) |
|---|---|
| *Staphylococcus epidermidis* | 44 (74.6) |
| *Staphylococcus aureus* | 11 (18.6) |
| *Staphylococcus haemolyticus* | 2 (3.4) |
| *Staphylococcus saprophyticus* | 1 (1.7) |
| *Streptococcus pneumoniae* | 1 (1.7) |

Table 3. Distribution of antibiotic resistance of isolated bacteria in dacryocystitis

| Antibiotics | Susceptible | Intermediate -resistant | Resistant |
|---|---|---|---|
| Chloramphenicol | 59 (100) | 0 | 0 |
| Ciprofloxacin | 59 (100) | 0 | 0 |
| Amikacin | 29 (49.2) | 0 | 30 (50.8) |
| Cefoxitin | 36 (61) | 0 | 23 (39) |
| Gentamycin | 24 (40.7) | 17 (28.8) | 18 (30.5) |
| Tetracycline | 29 (49.2) | 0 | 30 (50.8) |
| Erythromycin | 19 (32.2) | 0 | 40 (67.8) |

Austria, and Australia, *S. epidermidis* and *S. aureus* are the most frequent species isolated from dacrysistis [13-17].

In the current study, the most common organism isolated was *S. epidermidis* and *S. aureus* (74.6% and 18.6%, respectively) this result is comparable with other studies [1, 6, 17, 18]. However, this finding was much lower than the prevalence of Gram-positive bacteria in dacryocystitis recorded in Saudi Arabia (79.1 %), Finland (62 %), USA (68.8 %), Australia (54.4%), and in a previous study in Iran (68.8 %) [19-23]. In general, the most significant factors for differentiating the diversity of bacteria in different studies include variations in the number of patients, differences in the social status of patients, access to ophthalmology, and public health knowledge among individuals [6].

Operational intervention is the main treatment of nasolacrimal duct obstruction. Despite surgical procedures, the risk of infection of soft tissue increases fivefold without the use of antibiotics, suggesting the importance of antibiotics in the treatment of dacryocystitis [23]. On the other hand, resistance against antibiotics is a problem that affects the treatment of dacryocystitis. In recent decades, drug resistance has spread exponentially, which may be due to overuse and abuse of these medications [24]. In our study, Gram-positive organisms exhibited a high rate of sensitivity to chloramphenicol, vancomycin, and ciprofloxacin. This is in correlation with the studies of Kuchar *et al.* (2000), Kebede *et al.* (2010), Chung *et al.* (2019) also have documented effectiveness of chloramphenicol, and ciprofloxacin against Gram-positive bacteria [8, 23, 25, 26].

While *S. epidermidis* and *S. aureus* displayed the highest susceptibility to chloramphenicol and vancomycin, it should be noted that between 32% and 39% of antibiotic resistance to these two drugs has been recorded. Therefore the determination of bacterial species and the determination of drug sensitivity in patients with dacryocystitis appear to be significant [6].

In a study by Assefa *et al.* (2015) in Northwest Ethiopia, they found that the most susceptible antibiotics were nalidixic acid (87.1%), erythromycin (84.2%), ceftriaxone (95.3%), and gentamicin (83.3%) [18]. Briscoe *et al.* (2005) showed obtained bacteria have more sensitivity to ceftazidime (95%),

ciprofloxacin (86%), and cefuroxime (50%), respectively [27]. In the study by Shah *et al.* (2011) norfloxacin was the most effective antibiotic while penicillin showed the most resistant antibiotic [28]. The disparity between studies shows that because of the regional pathogens, there are obvious differences in the pattern of antibiotic resistance in the geographic areas [29].

The small sample size, and short period, were the limitations of this research. A larger research group with a longer study duration may provide a better result. Based on the results obtained, we have offered to direct alternatives for the selection of effective antibiotics for clinicians who take care of cases controlled for acute dacryocystitis diagnosis.

The most frequent bacteria isolated from acute dacryocystitis were *S. epidermidis* and *S. aureus* in our area. The most effective antibiotics against all isolated microorganisms from acute dacryocystitis include ciprofloxacin and chloramphenicol. These 'regional' results have significant public health consequences in this area of Iran for the treatment and prevention of dacryocystitis.

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**Author Contributions**

HE, and JF, study designed. AZ, and HE, carried out the experiments. HE, wrote the manuscript. FS, analyzed the results. JF, supervised the project. HE, FS, and, AZ, edited manuscript, submitted to the journal, and response to reviewers. All authors read and approved the final manuscript.

**Conflict of Interest**

None declared.

**Ethical declarations**

This study has the formal approval of the Research Ethics Committee of Isfahan University of Medical Sciences, Isfahan, Iran (Approval number: IR.MUI.REC.1396.3.203).

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