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Brief communication (Original)

Identifications of hordeolum pathogens and its susceptibility to antimicrobial agents in topical and oral medications

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Background: Unnecessary use of antibiotics can be a problem in the treatment of hordeolum since the providers prescriptions, pathogens and their susceptibilies are peculiar to local situations.
Objectives: We identified current pathogenic organisms in hordeolum and its susceptibilities to antimicrobial agents in topical eye medications in Thai patients.
Methods: Seventy-nine patients from the King Chulalongkorn Memorial Hospital who never received any treatment for hordeolum participated in the study. Pus specimens were collected from incision and curettage. The bacteria was stained by Gram stain and grown on aerobic and anaerobic culture agars. If there was bacterial growth, drug susceptibility test was conducted utilizing Ciprofloxacin, Fusidic acid, Oxytetracycline, Polymyxin, Neomycin, and Chloramphenicol.
Results: Bacterial growth was detected in 54 isolates from 50 patients (63.3%). These isolates were identified to be *Staphylococcus epidermidis* (19 isolates, 35.2%), *Proprionibacterium acnes* (13 isolates, 24.1%), *Staphylococcus aureus* (10 isolates, 18.5%), *Corynebacterium spp.* (10 isolates, 18.5%), *Aerococcus viridans* (1.85%), and *Prevotella intermedia* (1.85%). Susceptabilty test of *P. acne* to Tobramycin and Polymyxin showed MIC_{90} (Minimal Inhibitory Concentration) was more than 10 times lower compared to other antibiotics tested.
Conclusion: Most of the pathogens were from the normal skin flora. The most common organism continues to be the *Staphylococcus* species. All eye medications tested had antibiotic concentrations more than 10 times higher than the values of MIC_{90} except for Tobramycin and Polymyxin which indicated that there was an emergence of drug resistant *P. acne*.

Keywords: Hordeolum, Minimal Inhibitory Concentration (MIC), pathogen, susceptibility

Hordeolum is the most common, non-threatening eyelid disorder found in clinical practice worldwide resulting from an infected clogged sebaceous gland. It is usually self-limiting and will spontaneously disappear within one to two weeks [1-6]. Treatment for this disorder is very easy and can range from using warm compressions or antibiotics (topical antibacterial ophthalmic ointments, eye drops, and oral antibiotics) to incision and curettage (I&C). Even though this eye condition is universally found in the general population, yet currently there are no standard guidelines for treating hordeolum. As a result of this, opthalmologists and general practistioners tend to use a variety of antibiotics which can assist in the development of drug resistance pathogens or give rise to new species. From our previous study, we reported many Thai ophthalmologists' decision to use antibiotics solely at their discretion based on their prior experience [7] and available resources. Since there is scarce information on antibiotic usage, conclusions from literatures remain vague and controversial. For example, Fraunfelder FT would administer topical broad-spectrum antibiotics after I&C or in recurrent cases [8] whereas others believed systemic antibiotics should not be used at all unless there was significant cellutitis [1-4] otherwise local treatment should be of minimum, especially when antibiotic usage is of concern [6]. When there are many options available

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without any guidelines, physicians may prescribe unnecessary antibiotics which could give rise to drug resistant pathogens as well as increase side effects or serious adverse events for the patients.

Furthermore, certain bacterial species such as *Staphylococcus spp.* has been known to be difficult to treat because of its ability to develop resistance quickly [9]. Even though *Staphyllococcus spp.*, *Staphylococcus aureus* and *Staphylococcus epidermidis* [10], is the main organism involved in causing hordeolum, however it has been reported that in rare instances, other organisms such as *Staphylococcus saprophyticus, Diplococcus catarrhus*, *Moraxella sp.*, and *Trichophyton mentagrophytes* may also contribute to this eye disorder [10]. It is not known whether these other organisms can develop resistance as fast as *Staphylococcus spp.* and therefore vigilance is warranted when antibiotics are administered to patients. In addition, it has been shown that topical agents can induce resistance at extraocular sites compared to systemic antimicrobial agents [11, 12].

Even though there have been no reports or outbreaks of any drug resistance pathogens, we need to be careful in prescribing medications to patients with hordeolum. Increasing wide spread use of topical antibiotics is a growing concern. For this reason, we sought to identify the pathogenic organisms currently found in hordeolum and determine its susceptibility to antimicrobial agents in topical eye drops commonly used in Thai patients.

Materials and methods

Patients

Seventy-nine patients who had never been treated for uncomplicated hordeolum with an abscess formation larger than 5 mm and lasting for less than seven days were recruited from outpatient Department of Ophthalmology, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Patients were excluded from the study if they had a history of any antibiotics use when having hordeolum, a tendency to bleed, unable to have incision and drainage under local anesthesia, allergic to xylocaine or povidine, and have adjacent complications associated with hordeolum such as preseptal cellulites and blephalitis. Informed consent was obtained from each patient before entering the study. The trial was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Collection and Bacterial Culture Procedures, and Susceptibility Testing

The pus was collected under sterile technique during incision and drainage procedure. The pus was collected from the same incision site using several sterile swabs, and were immediately streaked onto three agar plates, smeared onto a glass slide, and inoculated into a tube containing thioglycolate broth. Cultures were performed on three different types of agars: Brucella agar for the growth of anaerobic bacteria, Blood agar for the growth of aerobic bacteria, and Chocolate agar for the growth of microaerophilic bacteria. The smeared slide was stained using Gram stain. Thioglycolate broth was used to ensure bacterial growth for those specimens containing very few amounts of pathogen. Anaerobic kit (Mitsubishi Gas Chemical Company, Inc, Tokyo, Japan) was used to grow anaerobic bacteria in an anaerobic environment. The kit is composed of an anaerobic bag, anaerobic clip, and Anaero Pouch- Anaero. Anaerobic Indicator was used to guarantee that the environment was anaerobic (Oxoid Ltd, Hants, UK). After the samples were collected, they were sent to the Microbiology Department, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Isolates obtained from the infected incision site were subjected to bacteriological examination using standard techniques and its strains were identified by its bacteriological culture and biochemical techniques. The isolates were also tested for antimicrobial susceptibility to Chloramphenicol, Fusidic acid, Tetracycline, Tobramycin, and Ciprofloxacin by using Etest (AB Biodisk, Solna, Sweden). Data was analyzed by using descriptive statistics.

Results

From a total of 79 patients, bacterial growth was observed in 50 patients (63.3%). A total of 54 isolates were detected. These isolates were identified and represented in **Tables 1 and 2**.

Staphylococcus epidermidis (19 isolates; 35.2%), *Proprionibacterium acnes* (13 isolates; 24.1%), *Staphylococcus aureus* (10 isolates; 18.5%), *Corynebacterium spp.* (10 isolates; 18.5%), *Aerococcus viridans* (1 isolate; 1.85%), and *Prevotella intermedia* (1 isolate; 1.85%). The Gram stain revealed the organism only 14 in 50 culture positive patients. The results from 13 specimens (92.9%) were consistent with the organisms from the culture specimens showed the character of the causative organism. Only one showed no growth from the culture.

From the antimicrobial susceptability testing, most of the isolates were susceptible to all antimicrobial agents tested with the exception of *Staphyllococus spp.* which had some resistance to Tetracycline. Most of the bacterial organisms had MIC_{50} and $_{90}$ values 10 times lower compared to the concentrations of Chloramphenicol, Fusidic acid, Tetracycline, Tobramycin, and Ciprofloxacin except for *Propionibacterium acnes* to Tobramycin and Polymyxin. This indicated that most antimicrobial agents were in the susceptible range for all isolates except for Tobramycin and Polymyxin. Concentrations of antimicrobial agents are presented in **Table 3**.

Table 1. Results of antimicrobial susceptibility tests in this study.

	N	Median (ug/ml)	MIC_{50} (ug/ml)	MIC_{90} (ug/ml)	Mode* (ug/ml)	MIC range (ug/ml)	% susceptible**
Staphylococcus epidermidis							
Chloramphenicol	19	2	2	128	2	2 - 256	78.95
Ciprofloxacin	19	0.125	0.125	0.302	0.125	0.064 - 32	94.74
Fusidic acid	19	0.125	0.125	1.4	0.125	0.047 - 256	89.47
Tetracycline	19	1.5	1.5	>256	>256	0.19 - >256	63.16
Tobramycin	19	0.125	0.125	0.3	0.125	0.064 - 256	94.74
Polymyxin	19	96	96	140.8	64	32 - 256	N/A
Staphylococcus aureus							
Chloramphenicol	10	3.5	3.5	6.6	3	0.75 - 12	100
Ciprofloxacin	10	0.315	0.315	1.075	0.19	0.19 - 4	90
Fusidic acid	10	0.094	0.094	0.25	0.094	0.047 - 0.25	100
Tetracycline	10	0.625	0.625	31.2	0.5	0.25 - 96	70
Tobramycin	10	0.44	0.44	0.55	0.5	0.25 - 1	100
Polymyxin	10	224	224	384	128	128 - 384	N/A
Corynebacterium spp.							
Chloramphenicol	10	1.75	1.75	17.6	1	0.75 - 32	N/A
Ciprofloxacin	10	0.047	0.047	3	0.047	0.023 - 3	N/A
Fusidic acid	10	1.5	1.5	2.6	2	0.25 - 8	N/A
Tetracycline	10	0.125	0.125	0.3	0.125	0.016 - 0.75	N/A
Tobramycin	10	0.38	0.38	1.1	0.25	0.125 - 2	N/A
Polymyxin	10	16	16	49.6	8	8 - 64	N/A
Propionibacterium acnes							
Chloramphenicol	13	0.5	0.5	2	0.125	0.125 - 2	N/A
Ciprofloxacin	13	0.125	0.125	0.25	0.25	0.016 - 0.25	N/A
Fusidic acid	13	1.5	1.5	2.8	1	0.25 - 3	N/A
Tetracycline	13	0.064	0.064	0.1188	0.064	0.032 - 0.25	N/A
Tobramycin	13	16	16	>256	>256	1 - >256	N/A
Polymyxin	13	192	192	>1024	>1024	12 - >1024	N/A

MIC_{50} = antibiotic concentration that would inhibit the growth of 50% of the tested bacterial isolates;
MIC_{90} = antibiotic concentration that would inhibit the growth of 90% of the tested bacterial isolates.
*The value among all observations that occurs at the greatest frequency.
**The Clinical Laboratory Standards Institute (CLSI) and the British Society for Antimicrobial Chemotherapy (BSAC) breakpoints for Staphylococcus spp.
N/A: No CLSI or BSAC breakpoints at this time

Table 2. Results of MIC of *Aerococcus viridans* and *Prevotella intermedia* in this study.

	Aerococcus viridians **(ug/ml)**	***Prevotella intermedia*** **(ug/ml)**
Chloramphenicol	1	1
Ciprofloxacin	0.25	0.5
Fusidic acid	16	1
Tetracycline	0.125	16
Tobramycin	3	>256
Polymyxin	384	1

Table 3. Concentration of antimicrobial agents in topical eyedrops.

Brand Name	Antimicrobial Agent	%	Concentration
Ciloxan	Ciprofloxacin	0.3	3.5 mg/ml
Tobrex	Tobramycin	0.3	3 mg/ml
Fucithalmic	Fusidic acid	1	15.7 mg/ml
Terramycin	Oxytetracycline	0.5	5 mg/g
	Polymyxin		10,000 U/g
Xanalin	Neomycin		1.75 mg/ml
	Polymyxin		5,000 U/ml
	Gradimicidin		0.025 mg/ml
Chloram	Chloramphenicol	0.5	5 mg/ml

Discussion

To our knowledge, this study is the first of its kind and represents an initial step in identifying all pathogens involved in the pathogenesis of hordeolum and its susceptibility to antibiotics used in the new era. A total of 29 cases out of 79 patients had no bacterial growth. We believe that for these cases, the hordeolum can be self-limiting or its occlusion lacks pathogen.

On the other hand, we detected bacterial growth in 54 isolates from 50 patients (63.3%). We identified 6 species of bacterial organism which were associated with hordeolum: *S. epidermidis*, *P. acnes*, *S. aureus*, *Corynebacterium spp.*, *Aerococcus viridans* and *Prevotella intermedia*. Most of them were organisms of the normal skin flora except for *Aerococcus viridans*. It is interesting to note that a decade ago, the most common pathogen in hordeolum was *Staphylococcus spp.* which included *S. aureus* and *S. epidermidis*. To this day, *Staphylococcus spp.* continues to be the dominant bacteria in this eyelid disorder. However, from this study, we were able to detect other organisms from the normal skin flora such as *P. acne* and *Corynebacterium spp.* at a higher incidence. These two organisms have not been reported as pathogens of hordeolum. We suspect that there may be an increase in its prevalence or perhaps the pus collection procedures and transfer techniques used today have improved allowing us to identify the pathogens more efficiently. In our study, the pus collection method was very accurate and proper bacterial culture procedures were followed. This allowed us to grow and have enough bacteria for identification.

From **Table1**, MIC_{90} values for *P. acne* to Tobramycin and Polymyxin were high; we found only one bacterial strain, *P. acne,* to be resistant to both Tobramycin and Polymyxin. Also, we found some *S. epidermidis* resistance to Teramycin (36.84%), a common antibiotic ointment used to treat hordeolum in Thailand. Other resistance strains were found in *Staphyllococcus spp.* to Tetracycline: 36.84% in *S. epidermidis* and 30% in *S. aureus*. Even though there were only two strains from *Staphyllococcus spp.* that were resistant to Tetracycline, we need to be careful in how antibiotics are dispensed because this organism is very resilient and has the ability to develop a variety of strategies in avoiding death. [10] This intermediate resistance to Tetracycline, an active ingredient in most of the common eye ointments available in the market, urgently requires doctors to heed to the concept of resistance. For example, *Staphyloccoci* is resistant to Methicillin [13, 14], Penicillin [15-17], Glycopeptides and Vancomycin [13] but back in 1944, it was resistant to only Penicillin. [18] This resistance was conferred in a relatively short period of time [18]. After developing resistance to Penicillin, *S. aureus* started to develop resistance to other antibiotics such as Nafcillin, Cloxacillin, and Dicloxacillin. Now the results obtained from this study were difficult to analyze because there were no interpretative criteria for external usage of antimicrobial agents. The interpretive criteria established by the Clinical and Laboratory Standards Institute (CLSI) and the British Society for Antimicrobial Chemotherapy (BSAC), as shown in **Table 4**, are based on serum concentrations and cannot be translated uniformly into in vivo efficacy for topical ophthalmic agents.

However, both interpretive criteria from CLSI and BSAC are typically used to determine the resistance of *Staphyllococcus spp.*, the most common causative organism of hordeolum. Another challenge we encountered in analyzing the results is whether the

Table 4. Interpretive criteria for the results of standard antimicrobial susceptibility test established by the Clinical Laboratory Standards Institute (CLSI) and the British Society for Antimicrobial Chemotherapy (BSAC) for Staphylococcus spp.

Agent	Susceptible	Intermediate	Resistant
Chloramphenicol	≤8	16	≥32
Ciprofloxacin	≤1	2	≥4
Fusidic acid*	≤1*,**		>1*,**
Tetracycline	≤4	8	≥16
Tobramycin	≤4	8	≥16
Polymyxin*			

* There are no CLSI interpretive criteria, ** Interpretive criteria by BSAC. There are no CLSI interpretive criteria for *Corynebacterium spp*, *Aerococcus viridans*, *Prevotella intermedia*, and *Propionibacterium acnes*

intraocular penetration of each antimicrobial agent is affected by the inflammation of the targeted site. We acknowledged that certain antimicrobial agents may have a better intraocular penetration compared to others [19-22]. It is also possible that sufficient drug levels may not be able to penetrate into the ocular mass to effectively inhibit growth of bacteria due to the inflammation at the infection site. In this study, we do not know the exact amount of antimicrobial agent that has entered into the ocular mass to ascertain the drug efficacy in the treatment of hordeolum without contributing to the rise of resistant strains. Hence, in order to interpret the susceptibility of the organism to the antimicrobial agents tested, we defined susceptible breakpoints using MIC values 10 times lower than the concentration of the antimicrobial agents used in the preparations.

From this study, we were able to identify the different types of pathogens involved in hordeolum and its susceptibility to antimicrobial agents tested. As for its clinical application, this data should be interpreted with caution as there are many factors and limitations that could affect the treatment of hordeolum: 1) ophthalmic vehicle [23], 2) pharmacokinetic of these antibiotics in the infected tissue [24], and 3) the responses of the human immune system. It is important to note that some antimicrobial agent may have an immunosuppressive action as seen with Fusidic acid [25].

Another potential limitation of this study is our sample size which was small due to our strict inclusion criteria; only those who have never received any treatment for hordeolum were eligible for the study. Because of this stringent criteria, it took us a total of five years to recruit 79 treatment-naive patients even though there were approximately 200 cases of hordeolum available per year. The reason for this is due to the fact that in Thailand, most patients usually will attempt to treat the disorder by themselves by buying antibiotic eye drops from the pharmacies. They will come to see the doctor only if the problem persists, has exacerbated to the point of extreme discomfort or has affected their quality of life. Moreover, since our hospital is a tertiary care institution, patients tend to come to our hospital as a last resort which usually indicates that they have undergone some form of treatment for hordeolum. We speculate that if our sample size was larger, we should be able to detect other bacterial species not previously reported and more significant numbers of resistant pathogens.

From this study, we were able to demonstrate that there was only that there was resistant to antibiotics from hordeolum isolate. With these results, we can only issue an early warning to doctors that there are other pathogens contributing to hordeolum with the potential to become resistant, cross- and/or multiple-resistant to antimicrobial agents if we continue to use them irresponsibly. From this study, we do not recommend the use of a single antibiotic agent to treat hordeolum, especially Tetracyclin, Tobramycin, or Polymyxin since resistant strains have been identified; a combination of antimicrobial agents should be used instead of monotherapies. Future studies are needed to assess the results of these topical antimicrobial agents in a clinical setting. An open, randomized trial utilizing PK/PD (pharmacokinetics/pharmacodynamics) and MIC in a bigger sample size is in the works. Overall, this information will have important implications for the clinical management of hordeolum and possibly preventing the emergence of resistant organisms.

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