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Microbiologic efficacy of 3-day treatment with azithromycin 1.5% eyedrops for purulent bacterial conjunctivitis

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PURPOSE. *Antibacterial efficacy of topically applied azithromycin 1.5% was compared with tobramycin 0.3% in a multicenter, randomized, investigator-masked study for the treatment of purulent bacterial conjunctivitis.*

METHODS. *A total of 1043 adults and children received either azithromycin twice daily for 3 days (n=524) or tobramycin every 2 hours while awake for 2 days, then four times daily for 5 days (n=519). Conjunctival swabbing was taken at days 0, 3, and 9, using alginate swabs resuspended in a dissolution-transport medium, providing rapid and reproducible results. Cagle's criteria were used to define the pathogenicity level for each isolated bacterium.*

RESULTS. *In the per-protocol set, the rate of bacteriologic resolution was 85.2% for azithromycin versus 83.8% for tobramycin on day 3, and 92.8% for azithromycin versus 94.6% for tobramycin on day 9. Azithromycin was demonstrated to be noninferior to tobramycin according to the 10% noninferiority margin. Although some bacteria were categorized as resistant to tested antibiotics, eradication was observed (for azithromycin: Acinetobacter, Enterobacteriaceae, Pseudomonas), highlighting the specific pharmacokinetics/pharmacodynamics of the ocular route.*

CONCLUSIONS. *In total, topical therapy with azithromycin 1.5% administered only twice daily for 3 days effectively eradicates most pathogenic bacteria associated with bacterial conjunctivitis. These microbiologic results are in accordance with the observed clinical outcome. This new anti-infective product has the advantage of a short treatment course which could lead to an improvement in patient compliance. (Eur J Ophthalmol 2008; 18: 858-68)*

KEY WORDS. *Topical azithromycin, Purulent bacterial conjunctivitis, Clinical activity spectrum, resistance, Bacterial microbiology, Short course treatment*

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INTRODUCTION

Purulent bacterial conjunctivitis is characterized by mucopurulent discharge and conjunctival hyperemia (1), occurring frequently in children or in patients with predisposing factors. It is a contagious ocular disease caused by infection with one or more bacterial species. Even if the mild cases are generally considered to be a self-limiting disorder, current consensus supports the use of topical antibiotics in purulent conjunctivitis (2-4): besides their clinical benefits, they hasten microbial remission and prevent spread of the infection (2-4). Delayed antibiotics seem to have reduced benefits for later microbiologic remission (5, 6), while a recent meta-analysis (7) demonstrated that topical antibiotics clearly provide significantly better rates of early microbiologic remission than placebo (3, 7, 8).

Purulent bacterial conjunctivitis is mainly caused by

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Gram-positive organisms, like *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococci,* especially *Streptococcus pneumoniae* (9, 10). The most common Gram-negative microorganism is *Haemophilus influenzae* (1, 11, 12). This distribution was found across the United States, various regions of Europe (AFSSAPS. Argumentaire. Collyres et autres topiques antibiotiques dans les infections oculaires superficielles. 2004; 1-27. http://afssaps.sante.fr/pdf/5/rbp/ophtarg.pdf acceded on December 2007) (13), and India, with the exception of *H influenzae,* which is recovered at lower rates in this latter country (10, 14). Although bacterial conjunctivitis can occur at any age, it frequently occurs in children. Among them, *H influenzae* and *S pneumoniae* are the most common pathogens and may be associated with epidemic occurrences of bacterial conjunctivitis (15, 16). *H influenzae* is also common in newborns and infants younger than 3 years.

The currently available topical antibacterials for ophthalmology globally present a short residence time and a short half life requiring frequent administrations (3 to 8 times a day) for treatment duration from 5 to 15 days, depending on the severity of the ocular surface infection. Azithromycin is a second generation macrolide which has a wide in vitro antimicrobial spectrum and pharmacokinetic properties well-adapted to the treatment of bacterial conjunctivitis, which is why it seemed interesting to take advantage of the molecule. One formulation of azithromycin 1% eyedrops had been already developed and is mainly sold in the United States, with a classic recommended duration of 7 days and a dosing varying according to the day of administration. In order to considerably shorten the treatment duration while maintaining the required antibacterial efficacy, a new topical formulation of azithromycin 1.5% eyedrops has been developed in Europe, Azyter® (T1225), with a simplified posology of twice a day during only 3 days.

Clinical efficacy of topical azithromycin 1.5% eyedrops has been demonstrated in the treatment of conjunctivitis due to *Chlamydia trachomatis* in a large clinical study involving 670 children with active trachoma (17). It is a time-dependent bactericidal antibiotic with a post-antibiotic effect (9, 18-24). Its good and prolonged bioavailability in tears and conjunctiva has been demonstrated after a single oral administration (25).

To tailor azithromycin pharmacokinetic properties to topical use in ophthalmology, five clinical studies were performed in healthy volunteers. The concentration of topical azithromycin 1.5% was chosen because it was shown to be safe and it reached higher concentrations in tears than azithromycin 0.5% and 1.0%. If instilled twice daily, azithromycin 1.5% was able to reach the usually accepted threshold for most Gram-positive and negative bacteria, based on the results of surrogate pharmacokinetic parameters in the tears. A 3-day twice daily treatment maintained a high azithromycin concentration for 4 days in tears and for 7 days in conjunctiva. This lighter dosage regimen would improve the treatment compliance, limiting patient premature treatment discontinuations and thus the risk of developing bacterial resistance.

The objective of this analysis was to compare the bacterial efficacy of topically applied azithromycin 1.5% to tobramycin 0.3% in the treatment of purulent bacterial conjunctivitis and to investigate the specific issues concerning the susceptibility of the bacterial isolates in the field of ophthalmology.

METHODS

Clinical methodology aspects of this study have been detailed in another article focused on the clinical results (26).

Study design

This is a multicenter, randomized, investigator-masked, parallel-group, noninferiority study. Samples were analyzed in blind conditions.

A total of 40 centers actively recruited patients in eight countries: France, Bulgaria, Guinea Conakry, India, Morocco, Portugal, Romania, and Tunisia.

The study was conducted in accordance with Good Clinical Practice, applicable guidelines, the Declaration of Helsinki, and local regulations. Ethics committee approval was obtained in each country prior to enrolling any patient. Written informed consent was obtained from all participants (or parent/guardian for patients under 18 years of age).

Patients

The study population included adults, children, infants, and newborns. Patients at least 1 day of age and diagnosed with purulent bacterial conjunctivitis (unilateral or bilateral) defined as bulbar conjunctival injection and conjunctival purulent discharge (mild, moderate, or severe) were eligible for inclusion.

Patients with bacterial conjunctivitis diagnosed ≥7 days ago; bacterial infection due to trauma or foreign body; dacryocystitis; corneal ulceration or keratitis; viral ocular infection; closed angle glaucoma; acute allergy conjunctivitis; clinically significant ocular abnormality; organic amblyopia, monophthalmia; corrected visual acuity below 20/100; contact lens; newborn (i.e., 0–2 months old) not born at term (<37 weeks of amenorrhea); ocular surgery, ocular laser treatment in last 3 months; systemic macrolide antibiotics in last month; topical (ocular, nasal, bronchial) treatments and/or systemic NSAIDs in the last day; and immunosuppressives and/or any systemic antibiotic on D0 were excluded from the study.

Study medications and dosing regimen

Patients were randomized to receive either azithromycin 1.5% eyedrops (T1225, Théa Laboratories) one drop twice daily for 3 days or tobramycin 0.3% eyedrops (Tobrex®, Alcon Laboratories), one drop every 2 hours while awake up to 8 times a day for 2 days, then one drop four times daily for 5 days.

Investigation schedule

Conjunctival swabbing was taken at day 0 (before treatment), day 3 (except for children aged <3 years), and day 9. Moreover, for a subset of consenting patients, an optional sampling was planned on day 28 for ancillary additional bacterial testing to determine the effect of treatment on the susceptibility of the bacterial isolates several weeks after treatment completion.

Collection of conjunctival specimens

Specimen collection was performed by the ophthalmologist investigator. A bacteriologic specimen was taken from each infected eye using an alginate swab passed along the cul-de-sac to the temporal margin and back to the nasal margin at least three times rotating the swab at 180°. The swab was introduced into the tube containing 2.4 mL of alginate-dissolution transport medium with calgon. Then, after vortexing, 0.5 mL of this transport medium were plated on Columbia-agar medium (with 5% sheep blood) and 0.5 mL were plated on chocolate-agar medium. Delay between sampling and plating had to be as short as possible.

Microbiological technical procedures

The bacteriologic analyses were performed by the local laboratory associated with the clinical investigation center.
The microbiologic procedure included methods for organism isolation, cultivation and cryopreservation, characterization, and identification and count of bacteria using standard morphologic methods.
For each type of colony, the micro-organism was counted if <300 CFU/plate and semiquantified as $\log_{10}$ if >300 colonies/plate.
In addition, for quality control purposes, isolates from transport media and pure cultures obtained from the original specimens were frozen, stored, and shipped to the Centralised Control Laboratory according to the appropriate freezing and shipment procedures.
In accordance with the European Guideline CPMP/EWP/558/95, the pre- and post-therapy susceptibilities of pathogens were assessed for patients with clinical failure at day 9. For these cases, additional microbiologic investigations were performed by the centralized laboratory. These cryopreserved isolates were reincubated and then tested for azithromycin and tobramycin susceptibilities by the E-test in blinded conditions (AB Biodisk).

Quality control procedures

Each local laboratory was validated by the Centralised Control Laboratory.
For validation of local laboratories, a random set of frozen bacteriologic specimens were tested by the centralized laboratory and the concordance between both results was analyzed.

Analysis of microbiologic results

To differentiate pathogen bacteria from the normal eye flora, validated thresholds based on colony unit counts were used. Cagle's microbiologic criteria were chosen as they are the most commonly used in the literature (27-33). These criteria were developed because bacterial conjunctivitis could be provoked by saprophytic bacteria. Cagle's criteria define the threshold between saprophytic and pathogenic level when cardinal signs of purulent bacterial conjunctivitis are present.
Cagle's criteria were slightly modified taking into account the actual pathogenicity level of the germs (Tab. I). All the germs were included in accordance with the original Ca-

gle's classification (29), with the exception of two germs with reduced pathogenicity (*Micrococcus luteus* and *Streptococcus viridans* or *alpha haemolytic*) for which more restrictive threshold based on the pathogenicity level of the bacteria was used (Tab. I).

The germs not expressly cited in Cagle's classification were classified according to their degree of pathogenicity. Their similarity to other germs was as follows:

Germs similar to streptococci (*Enterococcus*, *Aerococcus*, *Gemella,* and *Lactococcus*) were included in Group II (threshold: 10 CFU/mL)

Lactobacillus similar to *Corynebacterium* was included in Group IV (threshold: 1000 CFU/mL)

Stomatococcus mucilaginosus similar to *Micrococcus* were included in Group III (threshold: 100 CFU/mL)

At baseline, the determination of the causal bacteria isolated from cultures was based on the defined microbiologic threshold. A bacteriologic sample was positive when bacteria were isolated above the defined pathogenic thresholds.

At follow-up visits on days 3 and 9, bacteriologic resolution for patients with positive day 0 results was defined as the absence of bacteria or its reduction below pathogenic threshold for all bacteria isolated above the threshold at day 0.

Statistical analysis

In this noninferiority trial, analysis for efficacy was based on the per protocol (PP) set with culture positive at day 0. If both eyes were infected, the worse eye (or the right eye in case of equal severity) was chosen for statistical analysis.

TABLE I - MODIFIED CAGLE CLASSIFICATION

Organisms	Cagle's category	Pathogenic threshold (CFU/mL)
Gram positive		
Cocci		
Staphylococcus aureus	II	10
Staphylococcus epidermidis	III	100
Staphylococci coagulase negative (others)	III	100
Streptococcus pneumoniae	I	1
Streptococcus group A	I	1
*Streptococcus viridans or alpha haemolytic**	III	100
Streptococci (others)	II	10
*Micrococcus luteus/Stomatococcus mucilaginosus**	III	100
Cocci (others)†	II	10
Rods		
Corynebacterium	IV	1000
Bacillus	III	100
Gram negative		
Cocci		
Neisseria	I	1
Branhamella catarrhalis	II	10
Rods		
Haemophilus	I	1
Pseudomonas	I	1
Acinetobacter	I	1
Enterobacteriaceae‡	I	1
Gram negative rods (others)§	I	1

*Included into Cagle's Category III after decision of the bacteriologic committee.

†Aerococcus viridans, Enterococcus, Gemella haemolysans, Gemella morbillorum, Lactococcus lactis. They were included into Cagle's Category II after the decision of the bacteriologic committee.

‡Enterobacter agglomerans, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Morganella morganii, Proteus, Proteus mirabilis, Serratia marcescens.

§Aeromonas hydrophila, Alcaligenes faecalis, Burkholderia cepacia, Chromobacterium violaceum, Chryseobacterium indologenes, Flavimonas oryzihabitans, Stenotrophomonas maltophilia.

CFU = Colony-forming unit

Bacteriologic resolution rates at days 3 and 9 were considered to be secondary variables while the clinical rate at day 9 (test-of-cure visit) was defined as the primary endpoint (details published in another article (26)).

The statistical hypothesis was that azithromycin 1.5% eyedrops were noninferior to tobramycin with a noninferiority margin of 10%. Exact two-sided 95% CIs on the difference between the groups (azithromycin minus tobramycin) were calculated.

The required number of patients was 436, i.e., 218 evaluable patients with bacterial positive culture at day 0 per group.

RESULTS

Patient disposition

Among the 1043 randomized patients, 521 had positive cultures at day 0 (MITT set). Out of these 521 patients, 50 (25 in each group) had at least one major deviation (noncompliance with the study medication, randomization error, and/or discontinuation for other reasons not related to treatment) and 471 had no major deviation and were included in the PP set: 245 patients for azithromycin, 226 for tobramycin. Only 3.7% of patients discontinued the study from day 3 (Fig. 1).

Patient demographics and baseline characteristics

The overall mean ± SD age was 39.0±20.7 years, ranging from 4 days (newborn) to 87 years. There were 539 males (51.7%) and 504 females (48.3%) (Tab. II). There were no differences between groups at baseline.

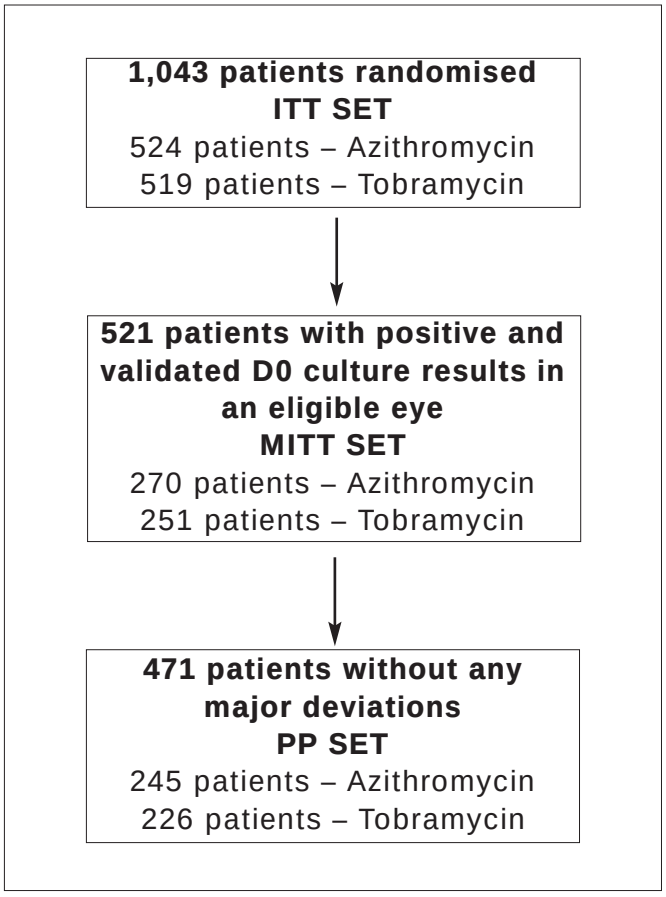

Fig. 1 - *Flow-Chart of patient sets.*

TABLE II - SUMMARY OF PATIENT DEMOGRAPHY (ITT SET)

Demographic variable	Summary of patient demography (ITT set)		
	Treatment groups		All patients
	T1225 (n=524)	Tobramycin (n=519)	ITT set (n=1043)
Gender			
Male, n (%)	263 (50.2)	276 (53.2)	539 (51.7)
Female, n (%)	261 (49.8)	243 (46.8)	504 (48.3)
Age, yr, mean ± SD	39.6±20.7	38.5±20.8	39.0±20.7
Age category			
0–27 d	3 (0.6)	2 (0.4)	5 (0.5)
28 d–23 mo	13 (2.5)	25 (4.8)	38 (3.6)
24 mo–11 yr	34 (6.5)	32 (6.2)	66 (6.3)
12–17 yr	23 (4.4)	18 (3.5)	41 (3.9)
18–64 yr	384 (73.3)	378 (72.8)	762 (73.1)
≥65 yr	67 (12.8)	64 (12.3)	131 (12.6)

Microbiology

Bacteriological status at baseline – At baseline, causative bacteria (Tab. III) were isolated above the pathogenic threshold as defined by the modified Cagle's classification in the 521 patients. The percentage of patients with positive culture was 51.5% in the azithromycin group and 48.4% in the tobramycin group.

The distribution of the most frequent causative bacteria was *S epidermidis* (39% of patients), other *Staphylococcus* coagulase negative (23%), *S aureus* (18%), *Haemophilus* (7%), *S pneumoniae* (6%), and Gram-negative bacteria: *Enterobacteriaceae* (6%) and *Acinetobacter* (5%). There were no notable differences in bacteria distribution between both treatment groups.

When considering countries, a slightly higher prevalence of *Haemophilus* was observed in France (27%) and Romania (17%). On the contrary, in India, a higher prevalence of *Acinetobacter* was reported (13%), whereas *Haemophilus* was isolated in only one patient.

In children, the bacteria distribution reported was slightly different when compared to the total number of patients. In children under 12 years old, a higher prevalence of *Haemophilus* (36%) and *S pneumoniae* (10%) was reported. This trend was confirmed by the prevalence of *Haemophilus* (50%) and *S pneumoniae* (15%) in children aged between 28 days and 24 months.

Bacteriologic resolution

The global rate of bacteriologic resolution in the PP set was 85.2% for azithromycin versus 83.8% for tobramycin on day 3, and 92.8% for azithromycin versus 94.6% for tobramycin on day 9. Azithromycin was demonstrated to be noninferior to tobramycin at both timepoints (Tab. IV). When considering bacteria genera or species, there were

TABLE III - DISTRIBUTION OF CAUSATIVE BACTERIA PER BACTERIAL CATEGORY IN THE WORSE EYE AT BASELINE (MITT SET)

Classification group		**Patients with positive bacteriologic samples at baseline, worse eye (MITT set), n (%)**		
		Treatment groups		
Organism	**Cagle's category***	**Azithromycin (n=270)**	**Tobramycin (n=251)**	**All patients MITT set (n=521)**
Gram positive				
Staphylococcus aureus	II	45 (16.7)	47 (18.7)	92 (17.7)
Staphylococcus epidermidis	III	105 (38.9)	96 (38.3)	201 (38.6)
Coagulase-negative Staphylococcus (others)	III	58 (21.5)	61 (24.3)	119 (22.8)
Streptococcus pneumoniae	I	19 (7.0)	11 (4.4)	30 (5.8)
Streptococcus viridans or alpha haemolytic	III	4 (1.5)	7 (2.8)	11 (2.1)
Streptococci (others)	II	8 (3.0)	4 (1.6)	12 (2.3)
Micrococcus luteus/ Stomatococcus mucilaginosus	III	3 (1.1)	2 (0.8)	5 (1.0)
Cocci (others)	II	10 (3.7)	8 (3.2)	18 (3.5)
Corynebacterium	IV	6 (2.2)	7 (2.8)	13 (2.5)
Bacillus	III	3 (1.1)	-	3 (0.6)
Gram negative				
Branhamella catarrhalis	II	1 (0.4)	1 (0.4)	2 (0.4)
Haemophilus	I	18 (6.7)	17 (6.8)	35 (6.7)
Pseudomonas	I	1 (0.4)	4 (1.6)	5 (1.0)
Acinetobacter	I	16 (5.9)	11 (4.4)	27 (5.2)
Enterobacteriaceae	I	15 (5.6)	17 (6.8)	32 (6.1)
Gram negative rods (others)	I	5 (1.9)	4 (1.6)	9 (1.7)

*Pathogenic threshold (CFU/mL): 1 for category I, 10 for category II, 100 for category III, and 1000 for category IV

no notable differences in the bacteriologic resolution rate on day 9 between both treatment groups. Results are summarized in Table V. After azithromycin treatment with the proposed dosage regimen, bacteriological resolution at day 9 ranged between 88% and 100% for the susceptible and moderately susceptible species. For resistant species, *Acinetobacter* were resolved for 80% of patients in both treatment groups; however, 100% of patients were clinically cured. The other isolated resistant strains (*Enterobacteriaceae*, *Pseudomonas*) were resolved for 100% of patients.

None of the prognostic factors (childhood, age category, disease severity at baseline) were found to have a significant effect on bacteriologic resolution rate when analyzed by logistic regression. Contrary to and as expected, relationship between bacteriologic resolution and clinical cure showed that the bacteriologic resolution rate was notably higher in patients with clinical cure (94.3% in azithromycin

TABLE IV - BACTERIOLOGIC RESOLUTION IN THE WORSE EYE ON DAYS 3 AND 9

Timepoint	No. (%) of patients with bacteriologic resolution in the worse eye (PP set)		Noninferiority analysis (Azithromycin minus Tobramycin)		
	Azithromycin (n=245)	Tobramycin (n=226)	Difference	Exact two-sided 5% CI on difference	Noninferiority*
D3†	202 (85.2)	181 (83.8)	1.4%	–5.3%; 8.3%	Accepted
D9‡	219 (92.8)	211 (94.6)	–1.8%	–6.6%; 3.0%	Accepted

*Based on noninferiority margin of –10% defined for primary efficacy variable.
†For azithromycin: n=237. For tobramycin: n=216. Remaining patients discontinued or missing data.
‡For azithromycin: n=236. For tobramycin: n=223. Remaining patients discontinued or missing data

TABLE V - BACTERIOLOGIC RESOLUTION IN THE WORSE EYE FOR BACTERIA ISOLATED ON DAY 0 (PP SET)

Classification category	Patient with bacteriologic resolution (n)/patients with some strain at day 0 and present at the visit, n (%)			
	T1225 (n=245)		Tobramycin (n=226)	
	Day 0	Day 9	Day 0	Day 9
Gram positive				
Staphylococcus aureus	42	38/40 (95)	45	43/44 (98)
Staphylococcus epidermidis	93	83/90 (92)	85	80/83 (96)
Coagulase-negative Staphylococcus (others)	53	47/51 (92)	57	52/57 (91)
Streptococcus pneumoniae	17	14/16 (88)	11	11/11 (100)
Streptococcus viridans or alpha haemolytic	4	4/4 (100)	7	7/7 (100)
Streptococcus (others)	6	5/5 (100)	4	4/4 (100)
Micrococcus luteus/Stomatococcus mucilaginosus	3	3/3 (100)	1	1/1 (100)
Cocci (others)	8	7/8 (88)	8	8/8 (100)
Corynebacterium	6	6/6 (100)	6	6/6 (100)
Bacillus	2	2/2 (100)	—	—
Gram negative				
Branhamella catarrhalis	1	1/1 (100)	1	1/1 (100)
Haemophilus	15	15/15 (100)	12	10/12 (83)
Pseudomonas	1	1/1 (100)	4	4/4 (100)
Acinetobacter	15	12/15 (80)	11	10/11 (91)
Enterobacteriaceae	14	14/14 (100)	15	15/15 (100)
Gram negative rods (others)	5	5/5 (100)	2	2/2 (100)

and 97.0% in tobramycin) than in patients without clinical cure (79.2% in azithromycin and 73.9% in tobramycin) in both treatment groups on day 9.

Among the 55 patients with clinical treatment failure and having attended the day 9 visit (29 in azithromycin group, 35 in tobramycin group), only two patients in azithromycin group and three patients in tobramycin group showed a non-resolution of the day 0 pathogen at day 9. Susceptibility profiles of these remaining bacteria were not altered after treatment as no shifts in MIC values for azithromycin or tobramycin were observed. A similar proportion of patients per group had another resistant bacteria detected at day 9: four patients in the azithromycin group and three patients in the tobramycin group.

Bacteriological results at day 28

To assess the impact of the treatment on the host flora, bacteriologic sampling was performed in a subset of patients on day 28. A total of 64 patients attended the day 28 Visit (29 in azithromycin group, 35 in tobramycin group); 62 had conjunctival sampling. No pathogen bacteria were isolated based on the clinical and microbiologic criteria. No notable impact of the treatment on the host flora was noted. No occurrence of bacterial purulent conjunctivitis due to resistant bacteria could be identified in this patient subset.

DISCUSSION

The purpose of this randomized study was to verify if the concept of 3-day treatment with azithromycin eyedrops could provide the antibacterial efficacy required for treating purulent conjunctivitis. The data presented in this article focus on the microbiologic results. The clinical efficacy data were part of a separate publication (26).

Methods of specimen collection and processing are essential for the isolation of bacteria. Specimen collection was performed using alginate swabs resuspended in a dissolution-transport medium providing more rapid and reproducible results (29). In addition, the bacteriologic analyses were performed just after sampling by a local laboratory close to the investigator center. Indeed, it was essential to perform the bacteriologic analyses locally in order to ensure the survival of the most fragile bacteria and to avoid any bacterial proliferation during the shipment responsible for wrong positive results. The methods used for transportation, cryopreservation, culture, identification, and susceptibility testing of the pathogens were validated by an independent laboratory and are also techniques commonly used in microbiology in the field of ophthalmology (34). In addition, a procedure was followed for the validation of centers with respect to the reliability of their local laboratories.

Another issue is the difficulty in considering the isolated organisms as an etiologic agent with a reasonable degree of certainty. For this reason, the quantitative method of Cagle (29) was chosen as it defines the threshold between commensal and pathogenic level (expressed in colony-forming units [CFU] per mL). These criteria are validated and are most often used in clinical trials for discrimination between nonpathologic bacterial flora and bacterial infection of the conjunctiva (27-33). They were slightly modified taking into account the actual pathogenicity level of the organisms. Two micro-organisms with reduced pathogenicity (*Micrococcus* and *Streptococcus* "others") were classified in a group with a lower pathogenicity (Group III; threshold: 100 CFU/mL) instead of the group defined by Cagle (Group II; threshold: 10 CFU/mL). Microorganisms not expressly cited in Cagle's classification were classified according to their similarity to other bacteria as described in the footnote of Table I. The purulent bacterial conjunctivitis was defined by microbiologically positive results at day 0 (i.e., bacteria were isolated above the pathogenic thresholds) as well as two cardinal clinical signs, i.e., combination of conjunctival bulbar hyperemia and discharge (35-38).

At baseline, the observed positive culture rate of around 50% was close to most of those reported in the literature (30, 38-41). Only a few authors reported more extreme values from 19% (40) to 86% (33). The bacteria distribution was consistent with the data reported in the published studies (16, 32, 35, 39, 42, 43). Staphylococci were the most frequently isolated bacteria. Then, the most commonly isolated strains were *Haemophilus*, *Enterobacteriaceae, S pneumoniae,* and *Acinetobacter*. In children under 12 years old, a higher prevalence of *Haemophilus* and *S pneumoniae* was reported.

The assessment of bacteriologic outcome (resolved/non-resolved) was restricted to causative organisms identified at baseline in patients with positive day 0 culture results. For statistical comparison between treatments, the 10% noninferiority limit was chosen in accordance with the literature (44-46). The results showed that azithromycin was noninferior to tobramycin at both timepoints. Both treat-

ments were highly effective with a bacteriologic cure rate of 85.2% for azithromycin versus 83.8% for tobramycin on day 3. Then, bacteriologic resolution increased to 92.8% for azithromycin versus 94.6% for tobramycin on day 9, i.e., 7 days after the end of treatment for azithromycin and 3 days for tobramycin. These findings are similar to those reported in the literature (16, 28, 30, 32, 35, 36, 38, 40). The Cochrane review performed by Sheikh and Hurwitz (7) showed that topical antibiotic treatment is associated with significantly better rates of early microbiologic remission than treatment with placebo. Similarly, better rates of late microbiologic remission are observed with antibiotic treatment than with placebo.

These microbiologic results supported the clinical cure outcome which showed noninferiority at day 9 with a significantly quicker clinical resolution at day 3 (clinical aspects of the study have been detailed in a previous publication (26)).

Our results at days 9 and 28 showed no changes in the susceptibility profiles of causative bacteria, and no acquired resistances were noted like those reported for the systemic use. For the bacteria known to present acquired resistance after oral route (official in vitro antimicrobial activity spectrum of azithromycin according to the French Authority for Medicinal Products, 2005), the bacteriologic resolution was high in the azithromycin group: this rate was equal to 92% for patients with *S epidermidis* and to 88% in case of *S pneumoniae*. In addition, bacteria known to be naturally resistant to azithromycin, such as *Acinetobacter*, *Enterobacteria,* and *Pseudomonas,* were eradicated for 100% of the patients by the topical azithromycin treatment BID for 3 days. The same phenomenon was observed with tobramycin for *Streptococcus* and *Enterococcus*.

These findings are consistent with the literature. In vitro antibacterial susceptibility tests do not always coincide with the clinical results, i.e., the in vivo activity spectrum (47). For azithromycin, this in vitro–in vivo paradox may be explained by some environmental factors like pH, and proteins present in vivo (48, 49). Such variation factors are described in the literature for other macrolides (50).

This discrepancy may also be explained by the fact that much higher levels are reached in tears (51, 52) and on ocular surface after local therapy than in the serum after systemic administration. Bacteria susceptibilities are precisely determined according to the serum levels (51, 52). Consequently, the concentrations achieved by ophthalmologic route are often sufficient to eradicate even organisms with high minimal inhibitory concentration (52). Clinical trials have already described that in vitro resistant bacteria could be eradicated by the topical formulation of antibiotics (32, 53, 54). Our results confirm those findings, showing that the spectrum of clinical activity of topical azithromycin 1.5% eyedrops in vivo is significantly widened compared to the in vitro susceptibilities to azithromycin.

Azithromycin 1.5% eyedrops provide the advantage of a required clinical and microbiologic efficacy level with low-frequency dosing regimen and short treatment course. This ease of use could lead to better patient compliance and consequently minimize the risk of favoring selection of resistant bacteria.

CONCLUSIONS

These microbiologic findings support the conclusion that topical therapy with azithromycin 1.5% BID 3 days effectively eradicates most pathogenic bacteria associated with bacterial conjunctivitis.

Azithromycin 1.5% belongs to the second generation of macrolides and represents a new antibiotic class in ophthalmology. It is packaged in six single-use containers, thus decreasing the risks of contamination and misuse. It provides the advantage of a required efficacy level with low-frequency dosing regimen and short treatment course, ensuring better patient compliance.

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This study was conducted in accordance with Good Clinical Practice, applicable guidelines, the Declaration of Helsinki, and local regulations. Ethics committee approvals were obtained prior to enrolling any subject. Written informed consent was obtained from each subject.

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