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ABSTRACT

Background Unpreserved single-dose unit (SDU) eye drops are commonly used to avoid benzalkonium chloride-related toxicity. Although intended for single use, many patients report off-label repeated use of SDUs over a prolonged period. We investigated whether repeated use of dexamethasone 0.1% SDUs in the same patient increases the bacterial contamination rate.

Methods We prospectively enrolled patients scheduled for inpatient corneal and glaucoma surgery receiving dexamethasone 0.1% SDU four times per day from the same vial. To assess contamination rates, one drop from the vial was cultured immediately after opening the SDU (t0), 10 hours later after four drop applications (t10) and 24 hours after opening without further drop applications (t24). Conjunctival swabs were taken before and after drop application. Contamination rate was assessed with a standard clinical culturing protocol without introducing a positive control.

Results 110 eyes of 109 patients were evaluated. Drops collected immediately after opening the SDU (t0) were contaminated in 9/110 cultures (8.1%). At t10, 13/110 cultures were contaminated (11.8%; p=0.267) and 11/110 at t24 (10.0%; t24 vs t0; p=1.00). In 5 of 21 cases of contaminated drops at t10 and/or t24, the same isolates were cultured from the initial conjunctival swab and the SDU. In three cases, the same bacterial species was found in consecutive samples.

Conclusion The contamination rate of the SDU did not increase after multiple use within 24 hours. Contamination from fingertip flora was more likely than from ocular surface flora. Reuse of dexamethasone 0.1% SDU in the same patient within 24 hours appears to be safe.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Unpreserved dexamethasone 0.1% single-dose unit eye drops are manufactured in non-reclosable vials but contain enough for multiple applications, with a risk of bacterial contamination.

WHAT THIS STUDY ADDS

⇒ The bacterial contamination occurred from fingertip flora after opening the vial and did not increase during an extended period.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE AND OR POLICY

⇒ Repeated administration of preservative-free dexamethasone 0.1% eye drops in the same patient appears safe. This leads to a reduction in the costs and carbon footprint of the treatment.

INTRODUCTION

Preservative-free eye drops enjoy high popularity for the treatment of a variety of ocular disorders in order to reduce benzalkonium chloride-induced corneal endothelial toxicity.[1 2] Single-dose units (SDU) are convenient for patients[3] and are widely used with artificial tears as well as ocular medications. Artificial tears preparations usually are reclosable and, therefore, repeatedly used over the course of a day. SDUs for ocular medications, on the other hand, are manufactured in non-reclosable vials but contain sufficient volume for the application of 5–10 drops. Many patients report repeated use of the SDUs with medications as they do with artificial tears while leaving them open at home. The multiple use of SDUs with medications would have the potential to significantly reduce costs, waste and the carbon dioxide footprint of the treatment.[4]

It is unclear, whether the off-label repeated administration of eye drops from the same SDU container reported by patients poses an infectious risk. Bacterial contamination of multiuse eye drops, even if they contain preserving agents, has been an ongoing concern[5] for regulatory agencies. Bacterial contamination rates of ophthalmic solutions range from 3% up to 35% in different settings.[6–10] Schein *et al* found a threefold increase of potentially pathogen microorganisms in the conjunctival flora among subjects under long-term drop, therapy postulating a contamination cycle between the eye-drop dispenser and the patient's conjunctiva.[11] Despite these theoretical concerns, keratitis associated with contaminated eye drops is exceptionally rare

with only 15 cases being reported worldwide over the past 40 years.[12–14] All these reported cases exclusively involved Gram-negative bacteria. However, the Centers for Disease Control and Prevention (CDC) has recently reported an outbreak of extensively drug-resistant *Pseudomonas aeruginosa* associated with artificial tears.[15]

The aim of this study was to assess the bacterial contamination rate of dexamethasone 0.1% SDU eye drops when used repeatedly over a 10-hour period and stored for 24 hours with an opened lid on the patient's bed side table. For this purpose, we studied bacterial cultures of the drop content at different time points in relation to the patients' conjunctival flora determined by conjunctival swabs.

MATERIALS AND METHODS

Study design

The prospective study was conducted at a Swiss ophthalmological tertiary referral centre.

Adult patients scheduled for elective inpatient cornea or glaucoma surgery were recruited consecutively from November 2015 to February 2018. Exclusion criteria were known ocular infectious disease, concurrent topical or systemic antibiotic treatment, and a known history of intraocular pressure increase due to steroids. Written informed consent was obtained from all patients. The study was approved by the local ethics committee (LU 13115, Ethikkommission Nordwest-und Zentralschweiz EKNZ) and Swissmedic (2014DR4109). Baseline clinical data including past ocular history, use of eye drops within the last 2 months, systemic antibiotic treatment within the last 2 weeks as well as any severe adverse event (SAE) occurring within 2 weeks of study inclusion were assessed using a questionnaire and hospital patient records. Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Sample size considerations

We assumed the contamination probability to be 7.0%. If the true probability of clinically relevant contamination was 1.0%, we would need 100 participants, that is, 14 cases of contamination and 2 cases of clinically relevant contamination to reject the null hypothesis that the OR equals 1 with probability (power) 0.8. The type I error probability associated with this test of this null hypothesis is 0.05.

Study procedure

All participants were treated with dexamethasone 0.1% eye drops manufactured in an SDU container (Dexafree 0.1%, Laboratoires Théa, F-63017 Clermont-Ferrand Cedex 2, France) four times on the day of hospital admission before surgery. The single dose vials contain 0.4 mL of preservative-free 0.1% dexamethasone (dexamethasone sodium phosphate) and excipients (edetate disodium, sodium chloride, disodium phosphate, aqua ad solutionem). For each patient, a separate vial was used. Drops from the vial were set aside for microbiological analysis before, during and after instilling the drops into the participant's eye. To open the vial, the plastic tip was twisted off manually. The vials do not possess any reclosing mechanism. In-between drop applications, the open vials were kept in a designated small medication cup at the patient's bedside at room temperature. Hand hygiene measures were performed before any drop application according to hospital guidelines. Conjunctival swabs were taken before the first and after the last drop administration on the consecutive day before surgery. For microbiological analysis, drops from the vial were placed on an agar plate at each time point by the ophthalmic nurse. The area for the drop placement was marked on the bottom of the plate with a pen.

The procedure was as detailed:

Day 1 (day of admission before surgery)

- First conjunctival swab by study physician.
- Opening of the SDU container by an ophthalmic nurse and immediate inoculation of a chocolate agar plate with a first drop of Dexafree 0.1% (t0).
- A total of four Dexafree 0.1% eye drop instillations from the same vial to the patient's eye between 11:00 and 21:00 by an ophthalmic nurse.
- Second inoculation of chocolate agar plate from the drop vial at the designated area after the fourth drop application (t10).
- The used drop vial is stored in the medication cup at the patient's bedside overnight.

Day 2 (day of surgery)

- Second conjunctival swab by surgeon prior to disinfection for planned ocular surgery.
- Third inoculation of chocolate agar plate from the drop vial on the designated area 24 hours after the vial had been opened without any further drop application to the patient by the ophthalmic nurse (t24).

All drop samples were directly inoculated onto chocolate agar and incubated at 37°C with 5% carbon dioxide for 48 hours allowing the growth of both Gram-positive and Gram-negative as well as nutritionally demanding bacteria. Conjunctival swabs were collected with a liquid Amies transportation medium (SwabAX, liquid Amies, Axon Lab AG, Baden, Switzerland) and then transferred onto chocolate agar plates in the laboratory. For the first 28 participants (dropouts: 8), the three drop samples were collected on the same agar plate and stored in a mobile incubator on the hospital ward prior to laboratory processing. During the study, the hospital introduced a new standard operating procedure, which forced us to revise the cultivation processes. Therefore, subsequent drops were placed on a separate chocolate agar plate and transferred directly to the laboratory for cultivation and analysis. For any timepoint, growth within the designated area (t0, t10, t24) was considered a drop contamination. Any growth outside of the designated area was not included in the analysis. Bacterial growth was identified

Table 1 Patient characteristics

Total eyes (n patients)	110 (109)
Female (n, %)	65 (59.6)
Mean age (years) (SD)	71.3 (11)
Right eye (n, %)	59 (53.6)
Primary diagnosis (n, %)	
Endothelial dysfunction	53 (48.2)
Glaucoma	49 (44.5)
Keratoconus/refractive error	6 (5.5)
Trauma	2 (1.8)
Use of eye drops in the previous 2 months (n eyes)	
1	31
2	36
3	15
>3	4

separately for each of the three designated areas on the agar plate and from the conjunctival swabs. Bacterial identification was performed with matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) using the Bruker MALDI Biotyper instrument. We defined clinically relevant contamination as presence of Gram-negative and facultatively pathogenic micro-organisms. Contamination with skin or mucous membrane flora was considered clinically irrelevant.

STATISTICAL ANALYSIS

We summarised continuous variables with means and SD. Dichotomous variables were summarised with percentages. To compare the contamination rate at different time points, we used the McNemar test. A p value less than 5% was considered statistically significant. Analyses were performed using the immediate command *mcci* of the Stata V.16.1. statistics software package. (StataCorp (2019) Stata Statistical Software: Release V.16. StataCorp LLC, College Station, Texas).

RESULTS

A total of 161 patients met the inclusion criteria and were enrolled in the study. Complete data sets were available of 110 eyes from 109 patients, which were included in the analysis. Dropouts were due to missing drop samples and swabs. One patient was enrolled two times consecutively, while one eye was included for each study entry. Table 1 gives an overview of baseline characteristics.

The rate of contaminated eye drops remained constant and was 8.1% for t0, 11.8% for t10 (p=0.267) and 10.0% for t24 (p=1.00). Some of the positive cultures showed multiple contaminants, thus the total number of isolates was 10 out of 9 contaminated cultures for t0, 18 out of 13 cultures for t10 (p=0.660) and 17 out of 11 cultures at t24 (p=0.217). Table 2 represents an overview of isolates cultured from the drops at different time points. A full list of all positive culture results is given in table 3. Coagulase negative staphylococci were by far most frequently detected. Of all drop contaminants, only two were Gram-negative isolates (*N. mucosa* and *M. osloensis*; table 3, number 108 and 125).

The first conjunctival swab before starting dexamethasone 0.1% drop application was positive from 33 eyes (30 %) and the second conjunctival swab on the next day after completing drop application before surgery was positive from 22 eyes (20%) (table 2). Gram-negative bacteria were detected in 27.3% of the first and in 18.2% of the second positive conjunctival swabs, respectively. In six cases, the first and second conjunctival swab revealed the same bacterial species (table 3).

In only 5 out of 21 cases of contaminated drops at t10 and/or t24, the same isolates were detectable in at least one of the eyedrop cultures as well as in the initial conjunctival swabs (table 3, number 16, 42, 68, 107, 118). One case had a drop contaminant (*S. epidermidis*) that was detectable in the second but not the first swab (table 3, number 76). The same type of bacteria was found consistently in consecutive drop samples in only three cases (table 3, number 16, 59 and 107).

SAE reported within 2weeks after study inclusion were postoperative hypotension (n=1), intraoperative hypotension (n=1), conjunctival leakage (n=1), cystoid macular oedema (n=1), endothelial dehiscence (n=2)

Table 2 Summary of positive culture results for swabs and drops (t0, t10, t24)

		Types of microorganisms identified						
	Positive samples	Total	CoNS	VGS	Enterococci	Gram negative	*S. aureus*	Other
Swab 1	33	41	23	0	3	8	2	5
T0	9	10	6	0	0	0	0	4
T10	13	18	12	1	1	0	0	4
T24	11	17	10	2	0	2	0	3
Swab 2	22	28	19	3	0	4	2	0

CoNS, coagulase negative staphylococci; VGS, viridans group streptococci.

Table 3 Isolates from conjunctival swabs and eye drops

Number	Conjunctival swab 1	Eye drops			Conjunctival swab 2
		t0	t10	t24	
16	*S. epidermidis*		*S. epidermidis*	*S. epidermidis*	*S. epidermidis*
31	*S. epidermidis*	–	*S. oralis, R. mucilaginosa*	–	–
32	*S. epidermidis* *S. aureus* *S. dysgalactiae* *E. faecalis*	–	–	–	–
34	–	–	–	–	*S. epidermidis* *S. capitis*
36	*S. aeruginosa*	–	–	–	–
37	*E. faecalis*	–	–	–	–
39	–	–	–	–	*M. osloensis*
42	*S. epidermidis* *S. aureus* *B. cereus*	–	*S. epidermidis* *B. cereus*	–	–
52				*S. capitis*	
53	–	*P. acnes*	*S. hominis*	–	*S. epidermidis*
56	*S. epidermidis* *P. vulgaris*	–	–	–	–
57	*S. hominis* *B. megaterium*	–	–	–	–
58	–	–	–	–	*S. epidermidis*
59	–	–	*S. epidermidis*	*S. epidermidis*	–
62	*W. falsenii*				
64	–	*S. epidermidis* *S. capitis*	–	–	–
67	*S. epidermidis*	–	–	–	–
68	*S. epidermidis*	–	*S. epidermidis*	–	–
70	*S. epidermidis*	–	–	–	–
71	*P. mirabilis*	–	–	*S. hominis* *K. palustris* *M. luteus*	*S. mirabilis*
74	–	–	–	–	*S. epidermidis* *S. aureus*
76	*A. radioresistens*	–	–	*S. epidermidis*	*S. epidermidis*
79	*S. epidermidis*	–	–	–	*S. epidermidis*
85	*S. hominis*	–	*S. epidermidis* *L. gasseri*	–	*S. aureus*
86	–	–	–	–	*S. epidermidis* *S. oralis-mitis*
87	*S. epidermidis*	–	–	–	–
91	*Kocuria spp.*	–	–	–	–
95	–	–	–	–	*S. hominis*
96	*Moraxella spp.*	–	–	–	*S. hominis*
98	–	–	–	*S. saprophyticus*	–
99	*M. osloensis*	–	–	–	–
102	*S. epidermidis*	–	–	–	–

Continued

Table 3 Continued

Number	Conjunctival swab 1	Eye drops t0	Eye drops t10	Eye drops t24	Conjunctival swab 2
107	*S. epidermidis* *E. faecalis*		*E. faecalis* *S. epidermidis*	*S. epidermidis* *S. schleiferi*	*S. haemolyticus* *S. oralis-mitis*
108	*E. cloacae*	*S. capitis*	–	*S. salivarius* *N. mucosa* *S. parasanguis*	–
109	*S. epidermidis*	*S. capitis*	–	–	–
110	*S. epidermidis*	–	*S. epidermidis*	–	–
114	*S. pasteuri*	*S. epidermidis*	–	*S. capitis*	–
117	*S. epidermidis*	–	–	–	*S. epidermidis* *S. oralis*
118	*S. capitis*	–	*S. capitis* *S. lugdunensis*	–	–
119	–	–	*S. caprae*	–	–
121	*S. caprae*	–	–	–	*S. caprae*
123	*S. epidermidis*	–	–	–	
125	–	–	–	*M. osloensis* *M. luteus*	–
127	–	–	–	–	*S. epidermidis*
130	–	*S. hominis*	–	–	–
131	*K. oxytoca*	–	–	–	*K. oxytoca*
132	–	–	–	–	*S. hominis*
135	*S. marcescens*	–	–	–	–
138	–	*B. cereus*	–	–	–
139	–	*B. cereus*	*Bacillus cereus*	–	*S. epidermidis*
142	–	*B. cereus*	–	–	–
152	–	–	–	–	*S. haemolyticus*
153	–	–	–	–	*S. epidermidis* *Moraxella spp.*
159	–	–	*S. epidermidis*	–	–
161	–	–	–	*S. oralis*	–
164	*S. epidermidis*	–	–	–	–

and intraoperative haemorrhage (n=1). We recorded no SAE related to ocular surface or intraocular infection.

DISCUSSION

This study found that opening SDU containers resulted in 8.1% bacterial contamination rate of the vial tip and the subsequently dispensed eye drop. Thereafter repeated use of SDU containers stored with an open tip on the patient's bedside table did not pose a risk for an additional contamination. Only 3 of the 110 SDUs demonstrated repeated positive cultures of the same bacterial species at different time points. This low rate suggests that bacteria do not readily replicate in the SDUs.

Our results are in accordance to previous studies that identified fingertip contamination as the main risk factor to eye drop container contamination.[16] In two in vivo studies, the contamination rate of preservative-free, singly or multiply used eye drops handled by patients or by nurses was found to be 2.0%–3.9%.[4 16] Much higher contamination rates of 17%–45% were detected when ophthalmic SDU were used multiple times on consecutive patients in an out-patient clinic setting.[17 18] Su *et al* have studied microbial contamination of different preservative-free single unit-dose ocular medications, including betamethasone, in an experimental set-up (ie, without patient contact) by instilling a drop onto an agar plate five times within 24 hours.[19] They concluded there was no air contamination of the drops up to 24 hours after opening. In contrast to previous studies testing the multiple application of eye drops in a single patient, our average contamination rate of 10.0% at different time points on opening the vial appears rather high, but other studies did not look at eye drops containing steroids. Indeed, unpreserved dexamethasone 0.1% was found to

have no relevant antimicrobial properties compared with other eye drops[20] and in-use steroid eye drops were the ones with the highest contamination rates in an extended care facility.[21] Moreover, inoculation of agar plates was performed by ophthalmic nurses not wearing face masks rather than in laboratory conditions, which may have increased the rate of contamination. Hence, the contamination rate in our study is likely to reflect the everyday contamination rate of SDU vials when used by patients in a home setting.

The conjunctival swabs revealed a mixed flora of Gram-positive and Gram-negative species as expected.[22–24] Our rate of positive conjunctival swabs (30% first swab and 20%s swab) was like the rate found by Bruttini *et al* (25.3%). However, other studies have found much higher rates of 82%–96.6%.[22 24] In our study, the second conjunctival swab was usually taken when the patient was already under general anaesthesia but prior to preoperative disinfection. This may explain the lower positive rate compared with the first conjunctival swab, as taking the swab in an awake patient is more prone to contamination by eyelid margin and lashes. In only one patient we found the same type of bacteria (*S. epidermidis*) in the drop and in the second but not first conjunctival swab, indicating a potential new drop-conjunctiva transmission. However, in the absence of genomic sequencing potential transmission remains elusive, as *S. epidermidis* is a common commensal.

During 2 weeks of follow-up, we did not record any adverse event related to ocular infectious disease, meaning the contaminants found in the eye drops did not even after repeated use lead to any clinically significant complication. While it can be argued that clinicians may be reluctant to change practice in high-risk patients such as those in the postoperative period, our data suggest that contamination in the 24-hour period after opening was no higher than immediately after opening the SDU, indicating that the procedure is safe.

For our study, we used a clinical routine culturing protocol for bacteria and fungi but did not introduce a positive control. Thus, the sensitivity and specificity of the testing system were not assessed. Moreover, we considered any contamination detected within the designated area on the chocolate agar plate as drop contamination, thus not differentiating between actual drop contamination and any contamination arising from handling the chocolate agar plate by the ophthalmic nurse. Performing the swab and application of eye drops was done according to best practice standards but was not supervised and recorded. We cannot rule out that differences in the application methods used across study participants influenced the detection rate of conjunctival swabs and likelihood of contamination. Despite these limitations, which potentially affect the magnitude of the contamination detected, our data suggest that the contamination rate does not increase after multiple uses compared with the newly opened SDU.

CONCLUSION

In conclusion, the risk of bacterial contamination of SDU eye drops primarily originates from the fingertip flora on the initial manual opening of the vial rather than from contamination through the ocular surface. As bacteria do not easily replicate in eye drop containers, repeated administration of preservative-free dexamethasone 0.1% eyedrops from a single-dose unit container over an extended period in the same patient appears safe, thereby reducing health-related costs, waste and the carbon footprint.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval The study was approved by the local Ethics Committee (Ethikkommission Nordwest- und Zentralschweiz EKNZ (LU 13115)) and Swissmedic (2014DR4109). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer-reviewed.

Data availability statement Data are available upon reasonable request.

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