

Bacterial Isolates from Contact Lenses, Frames and Their Susceptibility to Disinfectants

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Abstract— Bacteriological assessments of contact lenses and frames were determined using standard bacteriological methods and the susceptibility of the bacterial isolates to Clear Care Cleaning Disinfecting Solution (CCCDs), Eye-Look Optical Lens Cleaner (ELOC) and Hydrogen peroxide (H₂O₂) were assayed using disc diffusion technique. Bacterial isolates from the contact lenses and frames were: *Staphylococcus aureus*, *Streptococcus* spp, *Pseudomonas aeruginosa*, *Coagulase negative (CoN) Staphylococcus* spp, *Bacillus* spp, *Citrobacter freundii*, *Corynebacterium* spp, *Escherichia coli*, *Haemophilus influenzae*, *Micrococcus* spp. and *Aeromonas hydrophila*. Only 33 (55.0 %) contact lenses and 41 (68.3 %) frames swabs showed positive growth and of the 41 (68.3 %) frames' swabs with positive growth, 23 (38.3 %) showed growth of single bacterial isolate, 10 (16.7 %) showed growth of two bacterial isolates, while polybacterial growth was present in 7 (13.3 %). Only 25 (75.8 %) males' and 16 (59.3 %) females' frames swabs had bacteria growth, while contact lenses and frames from aged 21-30 yrs and 41-50 yrs had the highest and lowest numbers of bacteria colonization, respectively. The highest and lowest bacteria colonization of contact lenses and frames were from the farmers and civil servants, respectively. *Bacillus* spp BS-F13, BS-F57 and CoN-*Staphylococcus* spp CS-C1 were resistant to CCCDS, ELOC and H₂O₂. *P. aeruginosa* PA-C50 and *A. hydrophila* AH-C32 were resistant to both ELOC and H₂O₂. Only 2/18 (11.1%) and 5/18 (27.8%) of the Gram negative bacteria were resistant to ELOC and H₂O₂, respectively. The inhibitory zones obtained using CCCDS and ELOC ranged from 6.7±2.5mm to 12.8±0.5mm and 6.8±0.5mm to 11.3±0.8mm, respectively. Conclusively, this study has provided data on the bacterial isolates associated with contact lenses, frames and also showed the considerable variations in the antibacterial efficacy of contact lenses disinfection solutions.

Keywords— Bacteria, Disinfectants, Contact Lenses, Frames, Susceptibility.

I. INTRODUCTION

A contact lens is a piece of glass or similar transparent material with curved surface(s), shaped for use in optical instrument (Nwaugo *et al.*, 2008). Contact lenses are worn directly over the cornea mostly for correction of refractive error, improvement of visual acuity and enhancement of appearance for cosmetic or therapeutic reasons (Stern *et al.*, 2004). The lens makes images appear clearer and better when looked through with defective eyes (Eisenhart, 1985; Stern *et al.*, 2004; Nwaugo *et al.*, 2008). In 2004, it was estimated that 125 million people (2%) use contact lenses worldwide, including 28 to 38 million in the United States and the continuous increase in the use of contact lens may be because of its optical, occupational and cosmetic advantages to individuals. Contact lens wearers have increased in Nigeria, where the climatic conditions and the environment favour the growth of microorganisms. There may be more problems associated with contact lens wear in the developing nations than in the industrialized nations (Emina and Idu, 2011). The environment, type of contact lens, duration of wear, and type of contact lenses cleansing solutions have been reported as determinants of the microbial load on the contact lenses (Iskeleli *et al.*, 2002; Lee and Lim, 2003). Several authors have also reported that the introduction of contact lenses was associated with increase in ocular microbial complications (Devonshire *et al.*, 2003; Fleiszig and Evans, 2003). The adhesion and colonization of contact lenses by microorganisms, particularly bacteria have been implicated in several adverse events such as microbial keratitis (Willcox and Holden, 2001); contact lens related acute red eye (Szcotka-Flynn *et al.*, 2010); contact lens peripheral ulcer (Wu *et al.*, 2003) and infiltrative keratitis (Szcotka-Flynn *et al.*, 2010). Martins *et al.* (2002) also observed the presence of fungi, parasites and bacteria in contact lens swabs cultures. The occurrences of *Staphylococcus*, *Citrobacter*, *Aeromonas*, *Enterobacter* and *Pseudomonas* species on contact lenses have been reported (Sankaridurg *et al.* 2000; Brooks *et al.*, 2001). Some of these pathogenic organisms may be transferred quite easily from the contact lens, especially a hydrogel one, to the eye (Gondi, 1992; Gopinathan *et al.*, 1994; Wilhelmus *et al.*, 1998). Thus, efficient disinfection of the lens is essential. Disinfection allows elimination or destruction of bacteria, fungi and the inactivation of undesirable viruses (Garrigue, 1996). This capability is necessary in order to avoid severe ocular infections such as microbial keratitis and contact lens peripheral ulcer (Ishibashi, 1997; Wu *et al.*, 2003).

The chemical nature, application temperature and pH, concentration and quantity, contact time and tests method may determine the antimicrobial efficacy of the disinfectants (Russell and Hugo, 1987; Russell and Russell, 1995). Among the disinfectants used for cleaning contact lenses are hydrogen peroxides (H₂O₂), ELOC and Clear Care Cleaning Disinfecting Solution (CCCDs). Hydrogen peroxide is a lipid-soluble substance that produces highly reactive hydroxyl free-radical that attacks the lipid membrane, as well as the DNA, the mitochondria and other cell components. The toxicity of H₂O₂ to bacteria is mediated by this hydroxyl free-radical which is formed via the reaction of the oxidant with divalent iron (Russell and Hugo, 1987; Russell and Russell, 1995). Clear Care Cleaning Disinfecting Solution and Eye-Look Optical Lens Cleaner are peroxide-based clear care solutions that penetrate contact lenses and kills germs and bacteria.

The aim of the study was to investigate the antibacterial activities of disinfectants (H₂O₂, ELOC and CCCDS) on the bacteria isolated from contact lenses and frames.

II. MATERIALS AND METHODS

Study Population

The study was carried out from May to August, 2013. Sixty (60) participants in Uyo and Ikot Ekpene, aged ≤ 20 to ≥ 51 yrs were required to complete a questionnaire after seeking their consent. The questionnaire consisted of systematic questions regarding the age, sex, occupation, type of lenses and mode of disinfection of lenses.

Sterilization of Glass Wares

All the glass wares used for the research work were thoroughly washed with detergent and rinsed with clean water. The glass wares such as test tubes, Petri dishes, beakers, conical flasks, pipettes, Durham's tubes and McCartney bottles were sterilized using the hot air oven (Model DHG) at 180 °C for one and half hours. Wire loop was heat flamed to redness before and after use.

Collection and Bacteriology of Samples

Sixty (60) contact lenses and 60 frames were swabbed with sterile cotton swabs moistened with sterile normal saline solution. Each swab obtained was inoculated onto separate tubes with nutrient broth for 4-6 hr. These were gently streaked onto plates of Blood Agar, Chocolate agar, MacConkey Agar, Mannitol Salt Agar, Nutrient agar and Eosine Methylene Blue Agar and incubated at 37 °C for 24 hr. Cultures were considered negative if no growth was detected within 24-48 hr of incubation. Thereafter, the colonies were subcultured onto plates of nutrient agar and incubated at 37 °C for 24 hr. Pure cultures of isolates were streaked onto nutrient agar slants, incubated at 37 °C for 24 hr and stored in the refrigerator at 4 °C for characterization and identification. All isolates were Gram stained and subjected to various biochemical tests using standard methods (Holt *et al.*, 1994; Cheesbrough, 2006).

Preparation and Sterilization of Sensitivity Discs

Discs of 6 mm diameter were punched out using Whatman No. 1 filter paper with the aid of a paper punch and placed in Petri dishes. The Petri dishes containing the discs were sterilized in the hot air oven (Model DHG) at 180 °C for one and half hours, after which they were allowed to cool before used.

Susceptibility of the Bacterial Isolates to Disinfectants

The susceptibility of some randomly selected bacterial isolates from the contact lenses and frames to the disinfectants: Clear Care Cleaning Disinfecting Solution (CCCDs), Eye-Look Optical Lens Cleaner (ELOC) and Hydrogen peroxide (H₂O₂) were determined using disc diffusion method of Somchit *et al.* (2004). Mueller – Hinton Agar (MHA) was sterilized, cooled to 45 – 50 °C and then poured into sterilized Petri dishes. Sterile filter paper discs of 6 mm diameter were impregnated with each disinfectant and carefully placed onto each plate of Mueller – Hinton Agar which had previously been inoculated with 0.1 ml of bacterial isolate prepared directly from an overnight agar plate and adjusted to 0.5 McFarland Turbidity Standard using sterilized forcep. Each disc was sufficiently spaced out and kept at least 15 mm from the edge of the plate to prevent overlapping of zones. The plates were then incubated at 37 °C for 24 hr and zones of inhibition diameter (in millimeters) were determined using a ruler. The experiment was replicated thrice for each species and the mean zone of inhibition diameter (in millimeters) was determined in each case.

III. RESULTS

The percentage frequency of occurrences of the bacteria isolated from the contact lenses and frames are shown in Table 1. In contact lenses, *Staphylococcus aureus* had the highest frequency of occurrence 12 (22.6 %), followed by *Escherichia coli* 9 (17.0 %), *Streptococcus* spp 7(13.2 %), *Pseudomonas aeruginosa* 6 (11.3%), CoN-*Staphylococcus* spp 5 (9.4 %), *Bacillus* spp 3 (5.7 %), *Citrobacter freundii* 3 (5.7 %), *Corynebacterium* spp 2 (3.8 %), *Haemophilus influenzae* 2 (3.8 %), *Aeromonas hydrophila* 2 (3.8 %) and *Micrococcus* spp 2 (3.8 %). Of sixty-six (66) bacteria isolated from the frames, thirty-six (36) were Gram positive bacteria and thirty-two (32) were Gram negative bacteria. Among the Gram negative bacteria isolated from the frame, *E coli* had the highest percentage of occurrence, while *C. freundii* had the lowest percentage of occurrence (Table 1). The most predominant Gram positive bacteria from the frame was *S. aureus* 16 (23.5 %), followed by CoN-*Staphylococcus* spp and *Streptococcus* spp with 7 (10.3 %) each, *Corynebacterium* spp, *Bacillus* spp and *Micrococcus* spp had 2 (2.9 %) each (Table 1). Of the 60 swabbed obtained from the contact lenses, 33 (55.0 %) showed positive growth, while 27 (45.0 %) samples showed no growth in all the culture media used. Among the 33 samples with positive growth, 19 (31.7 %), 8 (13.3 %) and 6 (10.0 %) showed growth of single, two and three isolates, respectively (Table 2). Only 41 (68.3 %) of the swabbed obtained from the frames showed positive growth and 19 (31.7 %) samples showed no growth. Among the 41 samples with positive growth, 23 (38.3 %) showed growth of single bacterial isolate, 10 (16.7 %) showed growth of two bacterial isolates, while polybacterial growth was present in 8 (13.3 %) (Table 2). Contact lenses and frames of the males were more colonized by bacteria than that of the females (Table 3). In males, 20/33 (60.6 %) and 25/33 (75.8 %) of the swabs from the contact lenses and frames had bacteria growth, respectively, while between 48.1 % and 59.3 % swabs from the contact lenses and frames from the females had bacteria growth (Table 3). The highest number of contact lenses colonized by bacteria was obtained in subjects aged 21-30 yrs (73.3 %), followed by aged \geq 51 yrs (60.0 %), 31-40yrs (57.1 %), \leq 20 yrs (50.0 %) and 41-50 yrs (33.3 %). Of the 41 frames colonized by bacteria, 6 were from aged \geq 51 yrs, 10 from aged 31-40 yrs, 8 from aged 41-50 yrs, 13 from aged 21-30 yrs and 4 from aged \leq 20 yrs. Table 3 also shows the number and percentage of the contact lenses and frames colonized by bacteria in relation to the occupations. Highest numbers were obtained from the farmers with 9 (75.0 %) from contact lenses and 10 (83.3 %) from the frames while the lowest was from the civil servants with 4 (33.3 %).

TABLE 1
BACTERIAL ISOLATES FROM CONTACT LENSES AND FRAMES

Bacterial Isolates	Contact Lenses		Frames	
	Number	Percentages	Number	Percentages
	of Occurrences	of Occurrences	of Occurrences	of Occurrences
CoN- <i>Staphylococcus</i> spp	5	9.4	7	10.3
<i>Corynebacterium</i> spp	2	3.8	2	2.9
<i>Bacillus</i> spp	3	5.7	2	2.9
<i>Staphylococcus aureus</i>	12	22.6	16	23.5
<i>Pseudomonas aeruginosa</i>	6	11.3	8	11.8
<i>Streptococcus</i> spp	7	13.2	7	10.3
<i>Haemophilus influenzae</i>	2	3.8	6	8.8
<i>Aeromonas hydrophila</i>	2	3.8	4	5.9
<i>Citrobacter freundii</i>	3	5.7	3	4.4
<i>Micrococcus</i> spp	2	3.8	2	2.9
<i>Escherichia coli</i>	9	17.0	11	16.2
Total	53	100	68	100

Keys: CoN: Coagulase negative

TABLE 2
PREVALENCE OF MIXED BACTERIAL COLONIZATION OF CONTACT LENSES AND FRAMES

Source	No.(%) of Samples without Isolates	No.(%) of Samples with One Isolate	No.(%) of Samples with Two Isolates	No.(%) of Samples with Three Isolates	No.(%) of Samples with Four Isolates
Contact Lenses	60 27 (45.0)	19 (31.7)	8 (13.3)	6 (10.0)	0 (0.0)
Frames	60 19 (31.7)	23 (38.3)	10 (16.7)	7 (11.7)	1 (1.7)
Total	120 46 (38.3)	42 (35.0)	18 (15.0)	13 (10.8)	1 (0.8)

Values in parenthesis are percentages

TABLE 3
BACTERIAL COLONIZATION OF CONTACT LENSES AND FRAMES ACCORDING TO THE OCCUPATION / SEX AND AGE OF THE SUBJECTS

	Contact Lenses		Frames	
	No of Samples Collected	No (%) Infected	No of Samples Collected	No (%) Infected
Designation				
Farmers	12	9 (75.0)	12	10 (83.3)
Public Servants	12	6 (50.0)	12	8 (66.7)
Traders	12	6 (50.0)	12	10 (83.3)
Civil Servants	12	4 (33.3)	12	4 (33.3)
Students	12	8 (66.7)	12	9 (75.0)
Total	60	33 (55.0)	60	41 (68.3)
Sex				
Males	33	20 (60.6)	33	25 (75.8)
Females	27	13 (48.1)	27	16 (59.3)
Total	60	33 (55.0)	60	41 (68.3)
Age (Yrs)				
≤ 20	6	3 (50.0)	6	4 (66.7)
21-30	15	11 (73.3)	15	13 (86.7)
31-40	14	8 (57.1)	14	10 (71.4)
41-50	15	5 (33.3)	15	8 (53.3)
≥ 51	10	6 (60.0)	10	6 (60.0)
Total	60	33 (55.0)	60	41 (68.3)

TABLE 4
SUSCEPTIBILITY OF GRAM POSITIVE BACTERIAL ISOLATES TO DISINFECTANTS

Bacterial Isolates	Codes	Source	Zones of Inhibition (mm+S.D)		
			CCCDs	ELOC	H ₂ O ₂
CoN- <i>Staphylococcus</i> spp	CS-C1	CL	NS	NS	NS
	CS-C9	CL	8.7±1.7 ^{ab}	8.1±1.0 ^a	6.7±2.0 ^a
	CS-F1	FR	7.4±1.2 ^a	6.9±2.0 ^a	NS
	CS-F8	FR	8.3±0.5 ^a	7.5±0.5 ^a	NS
<i>Corynebacterium</i> spp	CB-C11	CL	7.9±2.0 ^a	7.2±0.5 ^a	6.9±1.6 ^a
	CB-C25	CL	9.0±1.0 ^{ab}	9.5±1.5 ^{ab}	7.7±1.0 ^a
	CB-F1	FR	7.8±1.2 ^a	8.3±1.2 ^a	NS
	CB-F5	FR	10.4±1.0 ^b	8.0±2.5 ^a	6.9±1.0 ^a
<i>Bacillus</i> spp	BS-C40	CL	6.7±2.5 ^a	NS	NS
	BS-F13	FR	NS	NS	NS
	BS-F57	FR	NS	NS	NS
<i>Staphylococcus aureus</i>	SA-C1	CL	9.1±1.6 ^{ab}	9.7±2.0 ^b	8.0±0.6 ^a
	SA-C32	CL	8.3±0.8 ^a	7.0±0.5 ^a	NS
	SA-C50	CL	9.5±1.0 ^{ab}	8.5±1.3 ^a	7.2±1.5 ^a
	SA-F42	FR	10.8±2.7 ^b	8.8±2.7 ^{ab}	8.1±2.0 ^a
	SA-F34	FR	7.0±1.5 ^a	7.4±1.0 ^a	NS
	SA-F6	FR	9.3±1.7 ^{ab}	7.4±2.5 ^a	6.8±1.5 ^a
<i>Streptococcus</i> spp	SS-C28	CL	NS	6.8±0.5 ^a	NS
	SS-C5	CL	9.9±1.3 ^b	10.1±1.5 ^b	8.4±1.0 ^a
	SS-C41	CL	10.1±1.5 ^b	10.9±0.5 ^b	8.7±0.5 ^{ab}
	SS-F36	FR	8.6±2.5 ^a	7.8±2.0 ^a	NS
	SS-F37	FR	7.3±1.0 ^a	6.9±2.5 ^a	6.9±0.5 ^a
<i>Micrococcus</i> spp	SS-C41	CL	8.4±1.3 ^a	9.9±1.3 ^b	7.8±0.5 ^a
	SS-F36	FR	11.8±0.8 ^b	11.0±0.5 ^b	9.3±1.0 ^b

Keys: CL: Contact Lenses; FR: Frame; NZ: No zone of inhibition; values in parenthesis are percentages; each inhibitory zone included 6 mm diameter of the disc., SD: Standard Deviation. Each value represents the mean of three replicates and standard deviation. Mean within the column followed by the different superscript letters are significant determined by Duncan's multiple range test ($P < 0.05$).

TABLE 5
SUSCEPTIBILITY OF GRAM NEGATIVE BACTERIAL ISOLATES TO DISINFECTANTS

Bacterial Isolates	Codes	Source	Zones of Inhibition (mm+S.D)		
			CCCDS	ELOC	H ₂ O ₂
<i>Pseudomonas aeruginosa</i>	PA-C32	CL	10.3±1.0 ^b	8.9±1.0 ^{ab}	8.2±1.5 ^a
	PA-C50	CL	7.1±1.0 ^a	NS	NS
	PA-F42	FR	7.0±2.5 ^a	7.1±1.3 ^a	NS
	PA-F6	FR	11.1±0.5 ^b	9.7±1.5 ^b	9.0±1.0 ^{ab}
<i>Haemophilus influenzae</i>	HI-C32	CL	12.8±0.5 ^b	10.3±1.2 ^b	9.3±2.5 ^b
	HI-C50	CL	11.4±1.7 ^b	9.1±0.5 ^{ab}	8.0±1.5 ^a
	HI-F42	FR	8.5±1.0 ^a	7.8±2.2 ^a	7.5±1.0 ^a
<i>Aeromonas hydrophila</i>	AH-C32	CL	7.4±1.0 ^a	NS	NS
	AH-C50	CL	9.7±1.5 ^b	7.3±1.5 ^a	NS
	AH-F42	FR	8.3±1.5 ^a	8.6±0.5 ^a	7.7±2.5 ^a
<i>Citrobacter</i> spp	CB-C32	CL	10.5±1.0 ^b	10.7±1.5 ^b	8.7±1.0 ^{ab}
	CB-C50	CL	9.5±0.8 ^b	9.1±1.2 ^{ab}	8.0±1.0 ^a
	CB-F42	FR	9.3±1.0 ^b	11.3±0.8 ^b	9.1±1.5 ^{ab}
	CB-F34	FR	8.6±1.6 ^a	7.0±1.6 ^a	NS
<i>Escherichia coli</i>	SA-C32	CL	9.9±1.2 ^b	9.4±1.0 ^{ab}	8.2±1.0 ^a
	SA-C50	CL	10.2±2.7 ^b	8.9±2.0 ^{ab}	8.7±1.3 ^{ab}
	SA-F42	FR	8.2±0.5 ^a	8.4±1.4 ^a	7.6±0.5 ^a
	SA-F6	FR	10.4±1.4 ^b	10.7±2.0 ^b	9.0±1.7 ^{ab}

Keys: CL: Contact Lenses; FR: Frame; NZ: No zone of inhibition; values in parenthesis are percentages; each zone included 6 mm diameter of the disc., SD: Standard Deviation. Each value represents the mean of three and standard deviation. Mean within the column followed by the different superscript letters are significant determined by Duncan's multiple range test ($P < 0.05$).

IV. DISCUSSION

Contact lens materials and consequently their physical properties have been modified substantially over the decades with the aim of providing clear vision with comfortable and safe lens wear. The adhesion and colonization of contact lenses by microorganisms, particularly bacteria have been reported Brooks et al. (2001). The strength of bacterial attachment is often influenced by their surface hydrophobicity. Organisms with greater surface hydrophobicity adhere in greater numbers than hydrophilic organisms. In this study CoN-Staphylococcus spp, Corynebacterium spp., Bacillus spp., S. aureus, P. aeruginosa, Streptococcus spp., A. hydrophila, C. freundii, E. coli and Micrococcus spp. were isolated from the contact lenses and frames. The occurrences of S. aureus, Citrobacter spp, A. hydrophila and Enterobacter spp in the contact lenses used in this study is in agreement with Sankaridurg et al. (2000) and Brooks et al. (2001). This study also confirms the previous results of Salha and Al-Zahrani (2012) who reported the occurrence of P. aeruginosa in the contact lenses.

P. aeruginosa is a ubiquitous environmental Gram negative bacterium, with a complex genetic makeup which enables its survival in a wide variety of nutritional environments and these characteristics contribute to the mechanisms by which it adheres to contact lenses. Cell surface hydrophobicity and appendages of P. aeruginosa participate in its adhesion processes (Sato and Okinaga, 1987; Hahn, 1997). The study carried out in Karachi by Rahim et al. (2008) showed the occurrence of Bacillus spp in contact lens and this study corroborates their reports. Our results also confirm the previous reports of Sankaridurg et al. (2000) who isolated Corynebacterium spp., Bacillus spp and S. aureus from the contact lenses.

Our results also showed mixed bacterial colonization in 23.3% contact lenses and 30.0% frames and this is in conformity with Emina and Idu (2011) who earlier reported that 22.4 % of the contact lenses of asymptomatic wearers in Lagos had mixed flora. The bacterial flora found in these contact lenses and frames of the asymptomatic wearers might be from the environment, water, physical contact, or from unhygienic habits of the wearers. In this study, contact lenses of 60.6 % males and 48.1 % females were contaminated with the bacterial flora. The slightly higher prevalence in males could be attributed to environmental influence as males are more outdoors than females (Nwaugo et al., 2008). Individuals of 21 - 30 yrs had the highest prevalence of bacterial contamination (73.3 %), followed by aged \geq 51yrs (60.0 %) while the least was aged 41-50 yrs (33.3 %). The highest prevalence of bacterial contamination in aged 21 - 30 yrs may be attributed to their activities and search of various economic ventures which could lead to contamination of their wears in the process.

The pathogenic bacteria may be transferred quite easily from the contact lens, especially a hydrogel one, to the eye. Thus, efficient disinfection of the lens is essential. The disinfection solutions (CCCDs, ELOC and H₂O₂) showed efficacy against all *Corynebacterium* spp, *S. aureus*, *Citrobacter* spp and *E. coli* isolated, while none of the disinfection solutions could prevent the growth of *Bacillus* spp BS-C57 and BS-F13. The disinfection solution (CCCDs) containing 3% hydrogen peroxide was the most effective against the Gram positive and Gram negative bacteria isolated from the contact lenses and frames in this study and this result is in agreement with Gondi (1992) that reported that 3% hydrogen peroxide was most effective against all micro-organisms. Hydrogen peroxide produces highly reactive hydroxyl free-radical that attacks the lipid membrane, as well as the DNA, the mitochondria and other cell components. The toxicity of H₂O₂ to bacteria is mediated by this hydroxyl free-radical which is formed via the reaction of the oxidant with divalent iron (Russell and Hugo, 1987; Russell and Russell, 1995).

Conclusively, this study has provided data on the bacterial isolates associated with contact lenses and frames and also showed the considerable variations in the antibacterial efficacy of contact lenses disinfection solutions.

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