MICROBIOLOGIC PROFILE OF FLEXIBLE ENDOSCOPE DISINFECTION IN TWO BRAZILIAN HOSPITALS

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ABSTRACT – ***Background*** - Endoscopes are routinely used in hospitals and clinics of the world and they can be potential sources of cross-infection when the decontamination process is unsuitable. ***Aim*** - The routines of flexible endoscope (bronchoscopes, esophagogastroduodenoscopes and colonoscopes) disinfection procedures used in two Brazilian university hospitals were evaluated during a 3-year period. ***Methods*** - Aleatory samples from internal channels of endoscopes were collected after patient examination and after cleaning/disinfection procedures. ***Results*** - A contamination >3 log10 was achieved in samples recovered from endoscopes after patient examination. These samples yielded gram-negative bacilli (n = 142: 56%), gram-positive cocci (n = 43: 17%), yeast cells (n = 43: 17%), and gram-positive bacilli (n = 26: 10%). Approximately, 72 out of 149 samples (48.32%) collected after undergoing the cleaning and disinfection procedures disclosed gram-negative bacilli (n = 55: 61%), gram-positive cocci (n = 21: 23%), gram-positive bacilli (n = 8: 9%) and yeast cells (n = 6: 7%). Esophagogastroduodenoscopes and colonoscopes were the most frequently contaminated devices. *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Enterobacter spp, Serratia marcescens, Proteus mirabilis, Citrobacter freundii, Staphylococcus aureus, Staphylococcus* coagulase negative, *Micrococcus luteus, Candida albicans, C. tropicalis, C. glabrata, C. guilliermondii, Bacillus* spp and *Corynebacterium* spp were predominantly identified. ***Conclusion*** - Inappropriate cleaning and low times of disinfection were respectively the major factors associated with the presence of microorganisms in colonoscopes and esophagogastroduodenoscopes. By analyzing the identified germs, hospital disinfection was considered of either intermediate or poor level. After this investigation, both university centers improved their previous protocols for disinfection and conditions for reprocessing endoscopes.

HEADINGS – Equipment contamination. Disinfection. Endoscopes.

INTRODUCTION

The importance of reliable sterilization or high-level disinfection of reusable items in patient care that come into contact with open wounds, sterile body cavities, the blood-stream, or mucous membrane surfaces has greatly increased awareness due to infectious diseases, as AIDS, viral hepatitis and tuberculosis, more prions speculations[5, 7, 16, 26]. Endoscopes are routinely used in hospitals and clinics and they are potential sources of cross-infection when cleaning and disinfection are not effective[1, 19, 24]. Organisms isolated from these devices have been associated with pseudoepidemic infections and outbreaks[2, 17, 21]. In São Paulo (Brazil), the evaluation through questionnaires sent to public, private, and philanthropic hospitals showed that cleaning and disinfection procedures of endoscopes were inadequately performed in 38 of 39 analyzed institutions[10]. Therefore, the reason for the present study within two university hospitals was the lack of microbiologic data on cleaning and disinfection of endoscopes in Brazil.

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MATERIAL AND METHODS

From 2000 to 2003, 298 samples collected from biopsy channel of bronchoscopes (n = 40), esophagogastroduodenoscopes (EGDs) (n = 138) and colonoscopes (n = 120) after patients examination and after usual decontamination procedures in two hospitals were analyzed. The cleaning of endoscopes was carried out with an enzymatic detergent solution, endozyme (LIFEZIME, Lab. Lifemed Medical Products Ltda). Manual disinfection was performed by soaking the device into 2% glutaraldehyde. The biopsy channel of bronchoscopes and EGDs was flushed thoroughly with 10 mL of sterilized phosphate buffered saline (PBS) by a disposable sterile syringe following clinical procedures and after the decontamination procedures. By using the same process, 20 mL of sterilized PBS were used in the colonoscope channel. Solutions were recovered in sterile tubes and immediately transported to the laboratory and processed. Homogenized 0.1 mL of each sample, and dilutions 1/10 and 1/100 in sterilized PBS, were inoculated onto three plates of 150 x 15mm respectively with tryptic soy agar (TSA), Sabouraud dextrose agar (SDA) with chloramphenicol, and MacConkey agar (Difco Laboratories). Residual glutaraldehyde was neutralized with sodium thiosulphate 0.6%. TSA and MacConkey plates were incubated at 37°C and observed after 24, 48 and 72 hours. SDA plates were incubated at 30°C and examined for 4 weeks. Random environmental specimens of rinse water were collected and cultured in the same media above mentioned. Different colony-types on each sample plate were selected for Gram stain characterization. The growth of each organism was quantified. Bacteria and fungi were identified according to classical techniques. Catalase production, growth in NaCl, esculin hydrolysis, Dnase and coagulase tests were carried out for the identification of gram-positive cocci (*Staphylococcus*, *Streptococcus* and *Enterococcus* spp). The *Enterobacteriaceae* family and non-fermentative bacilli were characterized by glucose oxidation and fermentation (O/F), oxidase, mobility, arginine hydrolysis, methyl red, indol production, lysine-deaminase, citrate, urea hydrolyses, production of H_2S and pigmentation, as pyoverdin and pyocyanin synthesis. EPM Mili and NF II (Probac, Brasil, BR) kits were used to presumptive identification of fermentative and non-fermentative bacteria, respectively. Gram strain, morphology, catalase production, presence and position of endospores identified the aerobic gram-positive bacilli. *Mycobacterium* spp contamination in centrifugates of samples collected from bronchoscopes was searched by bacilloscopy (BAAR). Fungi were initially characterized by their macroscopic and microscopic morphology. The biochemical profile of *Candida* spp was accessed through sugar assimilation and carbohydrate fermentation. *C. albicans* identification was performed by formation of germ tubes in plasma after 3 h at 37°C and chlamydospore production in Cornmeal agar at room temperature for 48 to 72 hours.

RESULTS

The manual disinfection of EGDs and colonoscopes at two Brazilian hospitals did not follow a suitable standard system (Table 1). Contamination of 10^3-10^6 cfu/mL was verified in samples recovered from endoscopes after patient examination. Colonoscopes, usually, presented microbial levels above 10^5 cfu/mL. Samples of endoscopes after using in patients yielded gram-negative bacilli (n = 142: 56%), gram-positive cocci (n = 43: 17%), yeast cells (n = 43: 17%), and gram-positive bacilli (n = 26: 10%). Approximately 48.32% (72 out of 149 samples) of the samples collected from the endoscopes after undergoing the cleaning and disinfection procedures had a microbial growth over 10^3 cfu/mL. EGDs were the most frequently contaminated devices (Table 2). Gram-negative bacilli (n = 55: 61%), gram-positive cocci (n = 21: 23%), gram-positive bacilli (n = 8: 9%) and yeast cells (n = 6: 7%) were found in decontaminated endoscopes. *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Enterobacter spp, Serratia marcescens, Proteus mirabilis, Citrobacter freundii, Staphylococcus aureus, Staphylococcus* coagulose negative*, Micrococcus luteus, Candida albicans, C. tropicalis, C. glabrata, C. guilliermondii, Bacillus* spp and *Corynebacterium* spp were predominantly isolated.

TABLE 1 – Characteristics of cleaning and disinfection processes used at the hospitals

Hospital instruments	n	Cleaning	Solution disinfection	Time (minutes)
Hospital-1				
Bronchoscope	10	e. s. + water	2% glutaraldehyde	15 – 30
EGDs	27	water	2% glutaraldehyde	2 – 5
Colonoscope	30	e. s. + water	2% glutaraldehyde	5 – 15
Hospital-2				
Bronchoscope	10	e. s. + water + us	2% glutaraldehyde	20 – 30
EGDs	42	e. s. + water	2% glutaraldehyde	5 – 10
Colonoscope	30	e. s. + water	2% glutaraldehyde	10 – 20

n = total number of collected samples
e.s.. = enzymatic solution
us = ultrasound

TABLE 2 – Microbial results of samples obtained after endoscope processing

Hospital instruments	Samples n	Contaminated		Samples X CFU/mL (TSA)
		n	%	
Hospital-1				
Bronchoscope	10	-	-	
EGDs	27	13	48.14	5.1 x 103
Colonoscope	30	21	70.00	3.8 x 106
Hospital-2				
Bronchoscope	10	-	-	
EGDs	42	23	54.76	2.4 x 103
Colonoscope	30	15	50.00	1.7 x 104
Total	149	72	48.32	

EGDs = esophagogastroduodenoscopes
n = total number of samples
CFU = colony forming units
TSA = tryptic soy agar

DISCUSSION

Hospital infections acquired by contaminated endoscopes are rare if suitable cleaning and high-level disinfection procedures are performed. Microbial resistance to biocides and establishment of biofilms are other important factors related with decontamination failure[22, 30]. Thus, the potential risk of nosocomial transmission of infections associated with the use of unsuitably decontaminated endoscopes should be always considered.

Currently, Brazilian manual or mechanical decontamination has involved enzymatic detergent cleaning and 2% glutaraldehyde disinfection. The National Health of Surveillance Agency (ANVISA) recommends disinfection time of 30 minutes with 2% glutaraldehyde[4]. However, we detected variations in the disinfection time, which is not conform with ANVISA recommendations (see Table 1). Similar problem was also related by COSTA[10], in 1996. In our study, short time of disinfection were found to be the major issues associated with the presence of microorganisms in EGDs. The positiveness of colonoscope samples should be related to inefficient cleaning process.

Contamination of medical devices can be due to environmental sources, patients or hospital staff[21, 29]. Random environmental specimens of rinse water collected showed that the main contamination source come from patients. Filtered water and 70% alcohol usually were used to rinse the endoscopes after disinfection.

Endoscopes contamination by mycobacteria, gram-negative bacilli and fungi is a well-recognized problem[11, 19, 24, 28]. MERIGHI et al.[23] recovered most frequently *Pseudomonas* spp, *Ps. aeruginosa* and *Staphylococcus* spp from both external and internal parts of EGDs and colonoscopes. An important cause of after-endoscopy infections was related to *Ps. aeruginosa*[2, 3]. ALVARADO et al.[3] reported an association of *P. aeruginosa* with sepses in individuals submitted to endoscopic examinations. In our investigation, *P. aeruginosa* was the most frequently microorganism isolated from endoscope samples and patients were the main source. Their predominance in processed endoscopes disclosed the inherent ability of this microorganism to resist enzymes and detergents with cationic and alkaline proprieties, besides their ability to form biofilm[8, 14, 18]. The high frequency of *K. pneumoniae*, *E. coli* and *S. marcescens* was also raised in EGDs and colonoscopes. Sometimes, polymicrobial contamination by such agents was seen. After decontamination procedure yeast cells have been more commonly isolated than moulds[17, 20, 27]. The transmission of uncommon yeast cells after unsuitably processed endoscope has been related to two cases of esophagitis by *T. asahii* in immunocompromized patients[20]. In this research, *Candida* species were cultured from EGDs unsuitably disinfected.

Some viruses may be present in both secretions and excretions (e.g.: sputum, gastric juice, saliva and enteric mucous) aspirated by endoscopes. HIV is easily eliminated of endoscopes by disinfection routine[15, 16]. However, errors in endoscope cleaning and disinfection increase the risk of HBV transmission[9, 12]. BÉCHEUR et al.[6] pointed out that instruments internal channel, where aspirated secretions go through, were the main site involved in HCV risk of cross-infection. RNA viral of HCV was reported in endoscopes decontaminated (29%) with 2% glutaraldehyde, suggesting that the transmission was possible after reprocessing[25]. *Helicobacter pylori* DNA was detected in 61% of endoscope samples from patients infected by this microorganism[13]. But, after disinfection for 20-30 minutes, the bacterial DNA was not amplified. By analyzing the identified germs, disinfection was considered of intermediate to low-level, especially for EGDs (Figure 1). In addition to the potential risk of transmission of the isolated species, other infectious agents such as *Helicobacter pylori, Mycobacterium tuberculosis*, hepatitis B and C viruses, which were not detected by classical identification methods could also be transmitted through endoscopes.

Failures in chemical decontamination procedures may occur in health centers of less developed countries where manual disinfection prevails. So, it is important that these results should be spread in

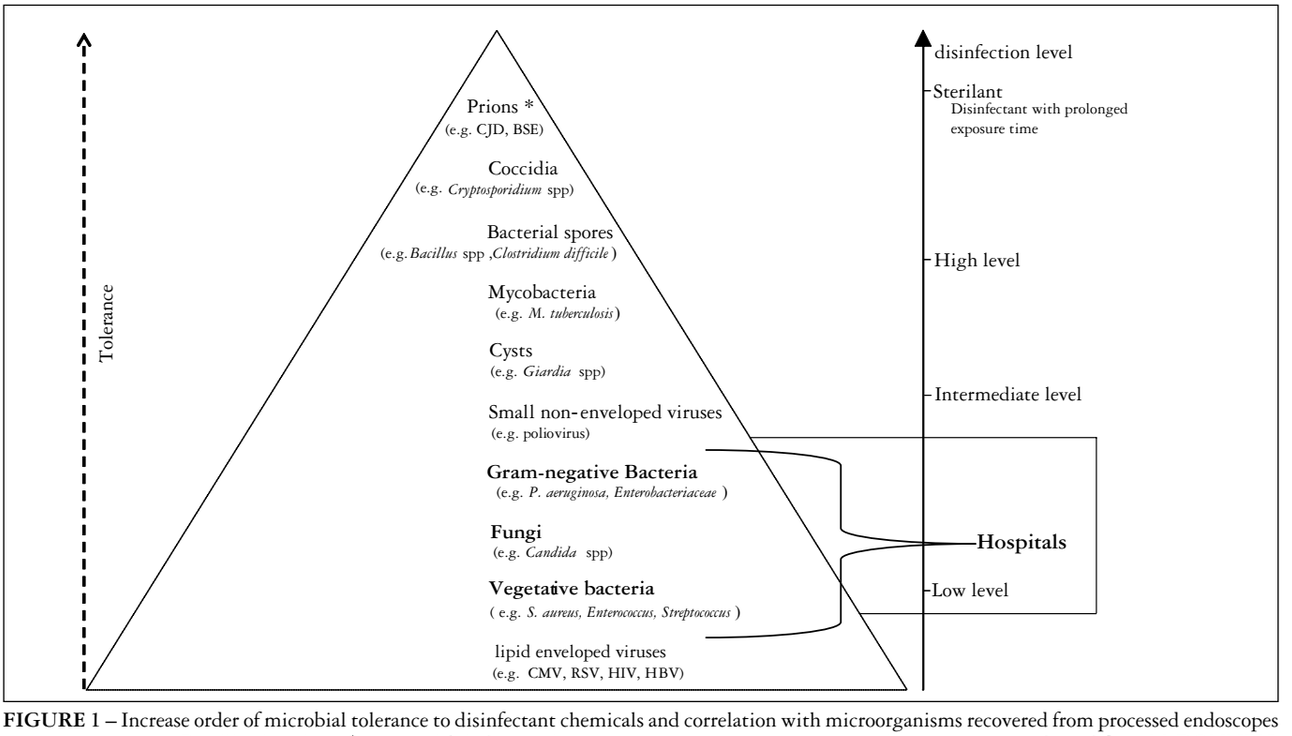

FIGURE 1 – Increase order of microbial tolerance to disinfectant chemicals and correlation with microorganisms recovered from processed endoscopes at two Brazilian hospitals. *The asterisk indicates that the conclusions are not yet universally agree upon. (Adapted from McDONNELL and RUSSELL[22]; WIDMER and FREI[30]

these countries, to call attention towards the importance of careful manual disinfection as well as the periodical bacteriological culture for monitoring disinfected endoscopes. The intense gastroscopy routine and the lack of familiarity with the correct use and action of biocides by health care professionals might have been the main causes of unsuitable cleaning and disinfection procedures. Technicians and endoscope users revealed interest on reviewing protocols after the investigation. Later on, significant changes occurred specially at the EGDs and colonoscope decontamination routine procedures followed within two Brazilian hospitals, in terms of increasing disinfection time and improving both facilities and staff conditions of endoscope cleaning and disinfection.

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RESUMO – ***Racional*** - Endoscópios são rotineiramente utilizados em hospitais e clínicas e podem ser fontes potenciais de infecção cruzada quando a descontaminação é inadequada. ***Objetivo*** - As rotinas de descontaminação dos endoscópios flexíveis (broncoscópios, gastrocópios e colonoscópios) realizadas em dois hospitais universitários do Brasil foram avaliadas durante 3 anos. ***Material e métodos*** - Amostras aleatórias foram coletadas dos canais internos dos endoscópios, depois que o aparelho era utilizado nos pacientes e após o processo de desinfecção. ***Resultados*** - Contaminação superior a 103 foi verificada em amostras coletadas após o exame endoscópico, sendo isolado bacilos gram-negativos (n = 142: 56%), cocos gram-positivos (n = 43: 17%), leveduras (n = 43: 17%) e bacilos gram-positivos (n = 26: 10%). Em 72 das 149 amostras coletadas após procedimentos de limpeza e desinfecção, detectou-se bacilos gram-negativos (n = 55: 61%), cocos gram-positivos (n = 21: 23%), bacilos gram-positivos (n = 8: 9%) e leveduras (n = 6: 7%). Gastroscópios e colonoscópios eram os aparelhos com maior freqüência e taxa de contaminação. *Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Enterobacter* spp, *Serratia marcescens, Proteus mirabilis, Citrobacter freundii, Staphylococcus aureus, Staphylococcus* coagulase negative, *Micrococcus luteus, Candida albicans, C. tropicalis, C. glabrata, C. guilliermondii, Bacillus* spp and *Corynebacterium* spp foram os mais identificados. ***Conclusão*** - A limpeza inapropriada e curto período de tempo de desinfecção eram, respectivamente, os maiores fatores associados com a presença de microrganismos em gastroscópios e colonoscópios. De acordo com os organismos isolados, considera-se que a desinfecção nos hospitais era de nível baixo a intermediário. Após a investigação, os centros de endoscopia adequaram seus protocolos, sanando os problemas verificados nos procedimentos de descontaminação dos endoscópios.

DESCRITORES – Contaminação de equipamentos. Desinfecção. Endoscópios.

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