Isabelle Cordeiro de Nojosa Sombra
(Organizadora)

DISCURSOS, SABERES E PRÁTICAS DA ENFERMAGEM 6

Atena Editora
Ano 2019

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APRESENTAÇÃO

A obra "*Discursos, Saberes e Práticas da Enfermagem"* aborda uma série de estudos realizados na área da Enfermagem, sendo suas publicações realizadas pela Atena Editora. Em sua totalidade está composta por 6 volumes, sendo eles classificados de acordo com a área de abrangência e temáticas de estudo. Em seus 27 capítulos, o volume VI aborda a publicações que envolvem aspectos relativos à variadas questões de Saúde Pública no Brasil nos diferentes níveis de atenção à saúde, desde a atenção básica até a assistência hospitalar.

Nesse contexto, a obra traz pesquisas sobre a assistência à diversas morbidades, sendo elas relacionadas ao aparelho cardiovascular, doenças infectocontagiosas, doenças crônicas, oncologia, além de estudos sobre dependência química, suicídio, acidentes de trânsito, dentre outros. Os estudos realizados contribuem para melhor entendimento acerca dos maiores enfrentamentos no que diz respeito a alguns dos principais problemas de Saúde Pública existentes no Brasil. Dessa forma, fornecem informações para elaboração de estratégias com finalidade de prevenção de doenças e agravos bem como para a promoção da saúde.

Portanto, este volume é dedicado aos profissionais atuantes nos serviços de saúde, com intuito de aprimorar seus conhecimentos e fornecer atualização de informações tão relevantes no cenário de Saúde Pública brasileiro. É dedicado também ao público usuário dos serviços de saúde, no tocante ao desenvolvimento de práticas de autocuidado, promoção da saúde e prevenção de agravos.

Ademais, esperamos que este livro possa fornecer informações relevantes para o fortalecimento e aprimoramento dos Programas de Saúde Pública vigentes no Brasil e, assim, melhorar cada vez mais os indicadores em saúde do país.

Isabelle C. de N. Sombra

SUMÁRIO

CAPÍTULO 1 1

CARACTERÍSTICAS SOCIODEMOGRÁFICAS DE PACIENTES COM TUBERCULOSE EM UM MUNICÍPIO DO AGRESTE ALAGOANO

Hidyanara Luiza de Paula
Amanda da Silva Bezerra
Viviane Milena Duarte dos Santos
Kleviton Leandro Alves dos Santos
Thayse Barbosa Sousa Magalhães
Ana Karla Rodrigues Lourenço
Bruno Barbosa da Silva
Italo Fernando de Melo
Joisse Ane Moreira da Silva Ferreira
Neíde Fernanda de Oliveira Silva
Sandra Mirthinielle Oliveira da Silva
Tamiris de Souza Xavier

DOI 10.22533/at.ed.7861923121

CAPÍTULO 2 5

IMPLANTAÇÃO DO ACOLHIMENTO E CLASSIFICAÇÃO DE RISCO NA ATENÇÃO BÁSICA:UM RELATO DE EXPERIÊNCIA

Kesia Jacqueline Ribeiro Oliveira
Camila Aparecida de Oliveira Alves
Herika do Nascimento Lima
Jenyffer Dias de Oliveira
Maria Da Glória Freitas
Cicera Alves Gomes
Anie Deomar Dalboni
Régina Cristina Rodrigues Da Silva
Silvana Pereira Gomes

DOI 10.22533/at.ed.7861923122

CAPÍTULO 3 11

ESTADO DEMOCRÁTICO: CONTRIBUIÇÕES DA ENFERMAGEM PARA GARANTIAS DOS DIREITOS Á SAÚDE PÚBLICA

Mleudy Layenny da Cunha Leite
Maria do Carmo Raposo

DOI 10.22533/at.ed.7861923123

CAPÍTULO 4 18

FOSFOETANOLAMINA EM FOCO: O QUE A MÍDIA DIVULGOU SOBRE O “MEDICAMENTO” PARA TRATAMENTO DO CÂNCER

Laura Beatriz Sousa de Jesus Martelletti
Graziani Izidoro Ferreira
Dirce Bellezi Guilhem

DOI 10.22533/at.ed.7861923124

CAPÍTULO 5 30

IMPACTO DOS EFEITOS COLATERAIS NA QUALIDADE DE VIDA RELACIONADA À SAÚDE DE PACIENTES COM LEUCEMIA EM TRATAMENTO QUIMIOTERÁPICO

Amanda Fonseca Baviera
Juliana Maria de Paula Avelar
Laís Reis Siqueira

Sterline Therrier
Camila Mendonça Lopes
Namie Okino Sawada

DOI 10.22533/at.ed.7861923125

CAPÍTULO 6 42

DOENÇAS RESPIRATÓRIAS E ALÉRGICAS E SUA ASSOCIAÇÃO A ÁCAROS DE AMBIENTE DOMICILIAR: ALGUMAS REFLEXÕES

Paula Michele Lohmann
Noeli Juarez Ferla
Guilherme Liberato da Silva
Paulo Roberto Vargas Fallavena
Arlete Eli Kunz da Costa
Camila Marchese
Gabriela Laste
Laura Roos
Jheniffer Otilia Costa

DOI 10.22533/at.ed.7861923126

CAPÍTULO 7 53

ESTUDO DAS ATIVIDADES FUNCIONAIS DE VIDA DIÁRIA EM PACIENTES COM DOENÇAS NEURODEGENERATIVAS RARAS

Vivian Susi de Assis Canizares
Naime Oliveira Ramos
José Juliano Cedaro
Andonai Krauze de França
Jorge Domingos de Sousa Filho
Cristiano Lucas de Menezes Alves
Jamaira do Nascimento Xavier
Thamyris Lucimar Pastorini Gonçalves
Thaynara Naiane Castro Campelo

DOI 10.22533/at.ed.7861923127

CAPÍTULO 8 64

ENFERMAGEM EM SAÚDE DA FAMÍLIA E A MENSURAÇÃO DA INCIDÊNCIA E INTENSIDADE DA DOR COMO QUINTO SINAL VITAL

Simone Regina Alves de Freitas Barros

DOI 10.22533/at.ed.7861923128

CAPÍTULO 9 77

ESTRATIFICAÇÃO DE RISCO PARA DOENÇAS CARDIOVASCULARES EM USUÁRIOS DO PROGRAMA HIPERDIA EM UMA ESTRATÉGIA SAÚDE DA FAMÍLIA

Nara da Silva Marisco
Guilherme Maidana Zanard
Graziani Maidana Zanardo
Giovani Sturmer
Kelly de Moura Oliveira Krause
Caroline Moraes Ferreira
Maicon Alves da Rosa

DOI 10.22533/at.ed.7861923129

CAPÍTULO 10 91

IDENTIFICAÇÃO DOS ASPECTOS DEMOGRÁFICOS E CLÍNICOS DE PACIENTES INTERNADOS EM UNIDADE DE CLÍNICA CIRÚRGICA

Marcella Gabrielle Betat

Arthur Saul Santiago
Miriam da Silveira Perrando
Márcia Aparecida Penna
Helena Carolina Noal
Lidiana Batista Teixeira Dutra Silveira
Rhea Silvia de Avila Soares
Tanise Martins dos Santos
Vera Regina Real Lima Garcia
Valdecir Zavarese da Costa
Suzinara Beatriz Soares de Lima
Alexsandra Micheline Real Saul-Rorato

DOI 10.22533/at.ed.78619231210

CAPÍTULO 11 101

INDICADORES MICROBIOLÓGICOS E FÍSICO-QUÍMICOS DO REPROCESSAMENTO DE ENDOSCÓPIOS FLEXÍVEIS: LIMPEZA MANUAL

Lissandra Chaves de Sousa Santos
Evandro Watanabe
Karen Vickery
Denise de Andrade

DOI 10.22533/at.ed.78619231211

CAPÍTULO 12 112

FATORES DE RISCO ASSOCIADOS À INSUFICIÊNCIA RENAL CRÔNICA

Claudio Roberto Farias Barbosa
Erlane Nunes de Andrade
Mariane Araújo Ramos
Maurício José Cordeiro Souza
Camila Rodrigues Barbosa Nemer
Marlucilena Pinheiro da Silva
Rubens Alex de Oliveira Menezes

DOI 10.22533/at.ed.78619231212

CAPÍTULO 13 126

ÍNDICE DE MORTALIDADE POR DOENÇAS DO APARELHO CIRCULATÓRIO E SUA RELAÇÃO COM OS DETERMINANTES SOCIAIS DA SAÚDE

Glauciely do Nascimento Pereira
Vânia Paula Stolte Rodrigues
Cátia Cristina Valadão Martins
Janaina Michelle Oliveira do Nascimento
Eluana Vieira da Silva

DOI 10.22533/at.ed.78619231213

CAPÍTULO 14 136

ÓBITOS POR LESÃO AUTOPROVADA NA FAIXA ETÁRIA DE 10 A 39 ANOS EM MATO GROSSO DO SUL

Jhonatan Ovando
Leilson Nunes Santana
Rafaela Palhano Medeiros Penrabel
Catia Cristina Valadão Martins Rosa
Vania Paula Stolte Rodrigues

DOI 10.22533/at.ed.78619231214

CAPÍTULO 15 144

NECESSIDADES DE QUALIFICAÇÃO DO PROCESSO DE TRABALHO DA ENFERMAGEM EM UTI PEDIÁTRICA

Francisco Rodrigues Martins
Francisco Hilângelo Vieira Barros
Antônia Gomes de Olinda
Mirelle Salgueiro Morini

DOI 10.22533/at.ed.78619231215

CAPÍTULO 16 151

O REGISTRO DA ASSISTÊNCIA DE ENFERMAGEM NO ACOMPANHAMENTO DA TUBERCULOSE

Marília Cattozatto dos Reis
Silvia Helena Figueiredo Vendramini
Anneliese Domingues Wysocki
Maria de Lourdes Sperli Geraldes Santos
Maria Amélia Zanon Ponce

DOI 10.22533/at.ed.78619231216

CAPÍTULO 17 163

O TRABALHO NA ESTRATÉGIA DE SAÚDE DA FAMÍLIA NA PERSPECTIVA DE MÉDICOS E ENFERMEIROS

Vera Gardênia Alves Viana
Maysa Ferreira Martins Ribreiro

DOI 10.22533/at.ed.78619231217

CAPÍTULO 18 176

LESÕES NO TRÂNSITO E USO DE EQUIPAMENTO DE PROTEÇÃO EM INDIVÍDUOS QUE SOFRERAM ACIDENTES ENVOLVENDO MOTOCICLETA

Jerusa da Silva Vaz
Adriana Alves Nery
Érica Assunção Carmo
Rafaela Almeida da Silva
Juliana da Silva Oliveira
Tatiane Oliveira de Souza Constâncio
Quézia Soares Oliveira

DOI 10.22533/at.ed.78619231218

CAPÍTULO 19 185

PERFIL DE USUÁRIOS DE UM CENTRO DE ATENÇÃO PSICOSSOCIAL ÁLCOOL E DROGAS II

Natália Hickembick Zuse
Leila Mariza Hildebrandt

DOI 10.22533/at.ed.78619231219

CAPÍTULO 20 198

MORTALIDADE POR ACIDENTES DE TRANSPORTE TERRESTRES EM MOTOCICLISTAS E AUTOMÓVEIS EM CAMPO GRANDE/MS

Edileuza Medina de Oliveira
Vania Paula Stolte Rodrigues
Rômulo Botelho Silva
Elaine Cristina da Fonseca Costa Pettengill
Cátia Cristina Valadão Martins Rosa

DOI 10.22533/at.ed.78619231220

CAPÍTULO 21 210

TRADIÇÕES, COSTUMES E VIVÊNCIAS RIBEIRINHAS HISTÓRICAS – UM OLHAR ATENTO PARA O OUTRO: UMA REVISÃO DA LITERATURA

Queren Hapuque Delaquila Machado Pedreira
Glaucia Valente Valadares
Fernanda Moreira Ballaris

DOI 10.22533/at.ed.78619231221

CAPÍTULO 22 221

TERAPIA COMUNITÁRIA COMO TECNOLOGIA DE CUIDADO DA ENFERMEIRA EM CENTRO DE ATENÇÃO PSICOSSOCIAL

Tâmara da Cruz Piedade Oliveira
Laís Chagas de Carvalho

DOI 10.22533/at.ed.78619231222

CAPÍTULO 23 233

PERFIL DE PACIENTES INTERNADOS EM UNIDADE HOSPITALAR DE CUIDADOS PALIATIVOS: UMA REVISÃO INTEGRATIVA DA LITERATURA

Maria Ancelma de Lima e Silva
Amanda Vilma de Oliveira Lacerda
Ana Carolina Oliveira de Freitas
Maiara Bezerra Dantas
Karina Ellen Alves de Albuquerque
Francisco Ayslan Ferreira Torres
Milena Silva Ferreira
Bruna Letícia Olimpio dos Santos
Sara Éllen Rodrigues de Lima
Adriana de Moraes Bezerra
Natana de Morais Ramos
Naanda Kaanna Matos de Souza

DOI 10.22533/at.ed.78619231223

CAPÍTULO 24 245

NECESSIDADES BÁSICAS AFETADAS E QUALIDADE DE VIDA EM HOMENS EM CIRURGIA ONCOLÓGICA

Ana Angélica de Souza Freitas
Maria José Coelho

DOI 10.22533/at.ed.78619231224

CAPÍTULO 25 256

O USO DE TERAPIA POR PRESSÃO NEGATIVA EM UM PACIENTE COM LESÕES POR PRESSÃO: UM ESTUDO DE CASO

Ana Paula de Magalhães Barbosa
Claudia Labriola de Medeiros Martins
Maria Lúcia Ferreira dos Santos Fernandes Filha
Rachel Cardoso da Silva
Rosemary Bacellar Ferreira de Lima

DOI 10.22533/at.ed.78619231225

CAPÍTULO 26 261

TERAPIA COM LASER DE BAIXA INTENSIDADE NA CICATRIZAÇÃO DE LESÕES POR PRESSÃO EM PACIENTE ORIUNDO DA UTI

Margarete Carréra Bittencourt
Rosana do Nascimento Rodrigues

Vanessa Diellen Pinto Ferreira
Anny Nayara Barros Garcia
Flavia Renata Neves Costa
DOI 10.22533/at.ed.78619231226

CAPÍTULO 27 276
RELAÇÃO ENTRE ATIVIDADE LABORAL E QUALIDADE DE VIDA EM PESSOAS COM PÉ DIABÉTICO
Aloma Renata Ricardino
Maria Gorette dos Reis
Marisa Dias Rolan Loureiro
DOI 10.22533/at.ed.78619231227

SOBRE A ORGANIZADORA 288

ÍNDICE REMISSIVO 289

CAPÍTULO 11

INDICADORES MICROBIOLÓGICOS E FÍSICO-QUÍMICOS DO REPROCESSAMENTO DE ENDOSCÓPIOS FLEXÍVEIS: LIMPEZA MANUAL

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RESUMO: A limpeza consiste em etapa fundamental para o reprocessamento de endoscópios gastrointestinais e prevenção de contaminação de pacientes. Este estudo teve como objetivo avaliar os níveis de contaminação orgânica e microbiológica de endoscópios gastrointestinais clinicamente utilizados. Utilizou-se testes de Adenosina trifosfato, polimerase C reativa quantitativa e cultura microbiana antes e após a limpeza manual dos endoscópios gastrointestinais utilizados em pacientes. A análise da limpeza manual demonstrou a eficácia na significativa redução de matéria orgânica e contaminação microbiana, com baixo percentual de batérias viáveis após o procedimento de limpeza.

PALAVRAS-CHAVE: endoscópios gastrointestinais; trifosfato de adenosina; controle de qualidade.

MICROBIOLOGICAL AND PHYSICOCHEMICAL INDICATORS OF FLEXIBLE ENDOSCOPE REPROCESSING: MANUAL CLEANING

ABSTRACT: Cleaning is a critial step on gastrointestinal endoscope reprocessing and patient contamination prevention. This study aimed to evaluate organic soil and microbial contamination levels of clinically used gastrointestinal endoscopes. For testing manual cleaning, it was used adenosine triphosphate assays, quantitative polymerase chain reaction and microbial culture, both before and after manual cleaning of clinically used gastrointestinal endoscopes. Endoscope manual cleaning analysis demonstrated the efficiency in reducing biological soil and microbial contamination, with a low percentage

of the sample with viable microorganisms.

KEYWORDS: gastrointestinal endoscopes; adenosine triphosphate; quality control.

1 I INTRODUCTION

Medical devices reprocessing consists of using a validated process to remove patient, microbial and non-organic contamination (KOLA, et al. 2015). The complexity of endoscopes makes their reprocessing challenging. As the reprocessing steps are susceptible to human error and due to the large bioburden present on endoscopes, the margin of safety in processing is small (HUMPHRIES; MCDONNELL, 2015), so each reprocessing step needs to be performed in a peerless manner to guarantee the validation of the process (AUMERAN, et al. 2010; RUTALA; WEBER, 2016).

Flexible endoscopes are reusable, semi-critical devices and as such are required to be cleaned and then subjected to either high level disinfection or sterilization to remove residual microbial contamination (CALDERWOOD, et al. 2018). Gastrointestinal endoscope contamination related to endoscopy procedures has been frequently published (VOOR, et al. 2018; EPSTEIN, et al. 2014; OFSTEAD, et al. 2015; GASTMEIER; VONBERG, 2014), including reports of transmission of multidrug resistant microorganisms (KIM, et al. 2016; MARSH, et al. 2015).

Endoscope contamination and healthcare associated infection (HAI) outbreaks associated with endoscopy have been due to a failure to adhere to reprocessing recommendations and/or equipment damage (KOLA, et al. 2015; ROBERTSON, et al. 2017; BAJOLET, et al. 2013; OFSTEAD, et al. 2010). However, infection related to the use of contaminated endoscopes has been reported even when endoscopes are reprocessed according to guidelines (OFSTEAD, et al. 2015; ENGLAND, et al. 2016).

Flexible endoscope reprocessing involves bedside cleaning, manual cleaning, disinfection/sterilization, drying and storage in an approved manner (WGO, 2019; CALDERWOOD, et al. 2018; SOBECC, 2017). It is well established that the cleaning step is critical for endoscope reprocessing success, as residual organic and inorganic matter within the internal channels of the endoscopes compromises biocide action (AGRAWAL; MUSCARELLA, 2011; BEILENHOFF, et al. 2017). Additionally, the presence of biofilms has already been proven to attach on endoscope internal channels (PAJKOS; VICKERY; COSSART, 2004) and, once formed, can compromise decontamination even if conducted rigorously under ideal conditions (REN-PEI, et al. 2014).

Given the difficulties involved in achieving successful endoscope reprocessing cited above, endoscope reprocessing evaluation can be helpful in detecting possible failures of the process, and thus decrease the possibility that inadequately processed

endoscopes are used for patient procedures. In this study, we aimed to evaluate organic soil and microbial contamination levels of clinically used gastrointestinal endoscopes before and after manual cleaning, utilizing adenosine triphosphate (ATP) assays, quantitative polymerase chain reaction (qPCR) and microbial culture.

2 I MATERIAL AND METHODS

Clinically used gastrointestinal flexible endoscopes (colonoscopes and gastroscopes) were assessed for organic soil by ATP bioluminescence and for microbial contamination by qPCR and microbial culture, before and after manual cleaning. Data was collected by the researcher on a endoscopy unit of a Hospital in Sydney, Australia. All endoscope reprocessing steps were performed by staff of the endoscopy unit as following described:

1) immediately after clinical use, bedside cleaning was done by wiping the external part of the endoscope with wipes soaked in enzymatic detergent and suctioning internal channels also with enzymatic detergent whilst still in the procedure room;

2) the endoscope was then transported to reprocessing dirty area for cleaning procedures where leak testing and manual cleaning were performed;

3) finally the endoscope was transported for disinfection/sterilization to the room housing the AER.

The external surface of the endoscope was sampled for ATP quantification by swabbing its distal end and the internal surface was sampled by a combination of brushing the biopsy channel and flushing each channel (air/water, suction and biopsy) with 10 mL of sterile water, followed by flush of air to collect sample. The flush from each channel and brush end, (removed using sterile scissors, were pooled. From the pooled sample a 20 μL aliquot was used for measurement of ATP levels, 100 μL was used for microbial culture and the remaining flush (approximately 30 mL) was used for DNA extraction and qPCR determination.

To test for differences in soil and microbial load contaminating cleaned and uncleaned endoscopes the Mann-Whitney, Wilcoxon and Spearman tests were conducted using IBM SPSS Statistics software version 23.0.

Adenosine Triphosphate (ATP) Testing

ATP was measured using the commercially available Hygiena ATP devices for endoscopes. The amount of ATP contaminating the external surface of the endoscopes was measured by swabbing the distal 30 cm external surface of endoscopes using Ultrasnap (Hygiena, Camarillo, Calif, USA); the ATP contaminating the internal channels was assessed using Aquasnap (Hygiena, Camarillo, Calif,

USA and using Endoswab (Hygiena, Camarillo, Calif, USA) to brush the distal section of the endoscope biopsy channel. All ATP assay devices were placed into the ATP bioluminescence device System Sure Plus (Hygiena, Camarillo, Calif, USA) according to manufacturer's instructions and the amount of contaminating ATP was measured as relative light units (RLU).

Microbial culture

The number of culturable bacteria was determined by pipetting 100 μL of endoscope channel flushes either neat sample or 100 μL of 10-fold serially diluted sample into horse agar plates (HBA) (Micromedia Laboratories, Victoria, Australia), applying the spread-plate technique and incubating at 37ºC for 24 hours. After the incubation period, the plates containing from 30 to 300 colonies were read and the number of colony forming units per milliliter (CFU/mL) was calculated.

Culture positive results were plated to obtain single morphology colonies. Isolated single colonies were subcultured onto Chromogenic UTI agar plates (Oxoid) using the streaking technique and incubated at 37ºC for a period of 18 to 24 hours prior to reading, followed by bacterial identification and subculture onto selective culture media including identification of multidrug resistant organisms (MDRO) (Figure 10). The selective media for MDRO identification included BrillanceTM MRSA agar plates (Oxoid, Thermo Fisher Scientific, Victoria, Australia) with 99.7% specificity and 95.4% sensitivity for Methicillin-resistant *Staphylococcus aureus* (MRSA) detection; Brilliance ESBL agar (Oxoid, Thermo Fisher Scientific, Victoria, Australia) with 95% sensitivity and 94% selectivity for detection of extended-spectrum beta-lactamase (ESBL) producing gram-negative bacteria; and BrillianceTM VRE agar (Oxoid, Thermo Fisher Scientific, Victoria, Australia) with a sensitivity of 94.7% at 24 hrs, and 100% sensitivity with 100% specificity at 48 hrs for Vancomycin-resistant *Enterococcus* (VRE) detection. For Brilliance ESBL agar plates readings the following sequence was used: coloured colonies were confirmed to be ESBL-positive, *E. coli* colonies were pink or blue in colour; while *Klebsiella*, *Enterobacter*, *Serratia*, and *Citrobacter* colonies were coloured green.

DNA extraction and quantitative PCR

For bacterial load determination, DNA was digested from inside the endoscope channels by syringing 2 mL of digestion mix (50nM Tris/HCl pH 7.5; 150nM NaCl; 2 mM EDTA; 1% SDS; proteinase K 20mg/mL) inside a 20 cm section of channel and incubating at 56 °C overnight. The channel section was then sonicated at an average of 43 mHz in ultrasonic bath (Soniclean, JMR Australia) for 20 minutes and the sonicate collected into a clean tube. The proteinase K was inactivated by heating to 95 °C, cooled and 200 μL of 10 mg/mL lysozyme (Sigma-Aldrich, Castle Hill,

Australia) added followed by incubation at 56 °C for 2 hours.

DNA was then extracted using the salt precipitation method. Briefly, 640 μL of 5M NaCl was added to the digested sample, mixed vigorously and cooled on dry ice for 10 minutes prior to centrifugation at 2500 rpm at 4 ºC for 15 minutes. The supernatant was transferred to a new tube before centrifuging at 2500 rpm at 4 ºC for 15 minutes and the new supernatant transferred to new tube prior to overnight alcohol precipitation at -20 ºC and centrifugation. DNA pellets were resuspended in 50 μL buffer (10mM Tris/HCL pH 8.0, 1mM EDTA).

Contaminating bacterial numbers were determined by real-time quantitative PCR using 16S rRNA Eubacterial universal primers 341F 5´- CCTACGGGAGGCAGCAG-3` and 534R 5`-ATTACCGCGGCTGCTGG-3` as described previously (JACOMBS, et al. 2012).

3 I RESULTS

A total of 99 clinically used endoscopes (63 colonoscopes and 36 gastroscopes) were tested both before and after manual cleaning. For determining amount of residual soil or dirtiness the amount of ATP contaminating both the external and internal part of the endoscopes were measured separately. Gastroscopes had significantly higher ATP values than colonoscopes both internally ($p<0.001$) and externally ($p<0.001$) after manual cleaning (Figure 1).

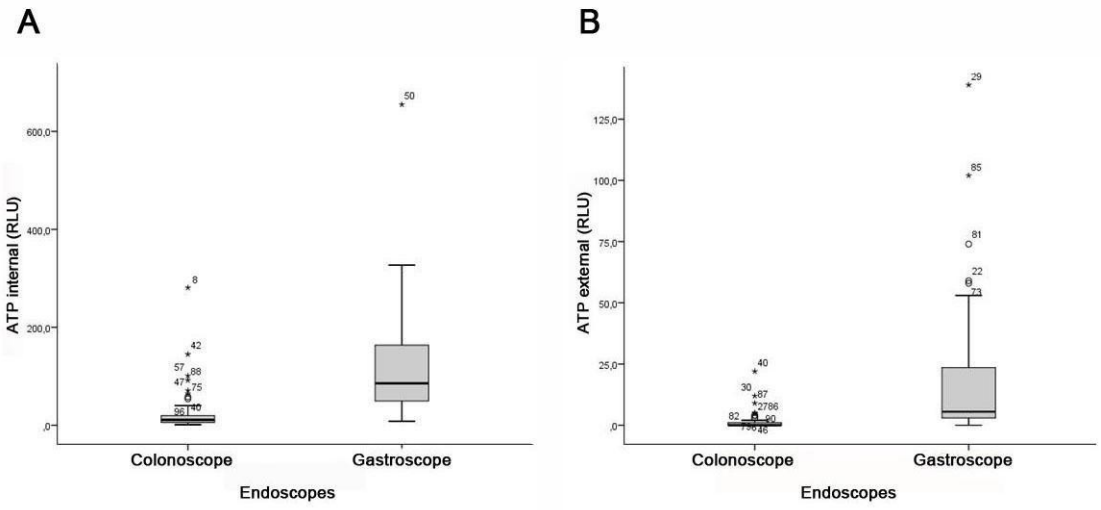

Figure 1. ATP values distribution by endoscope type after manual cleaning tested internally (A) and externally (B).

Manual cleaning of gastroscopes resulted in a significantly greater reduction of ATP contamination than that seen with colonoscopes both internally ($p<0.001$) and externally ($p<0.001$) (Figure 2).

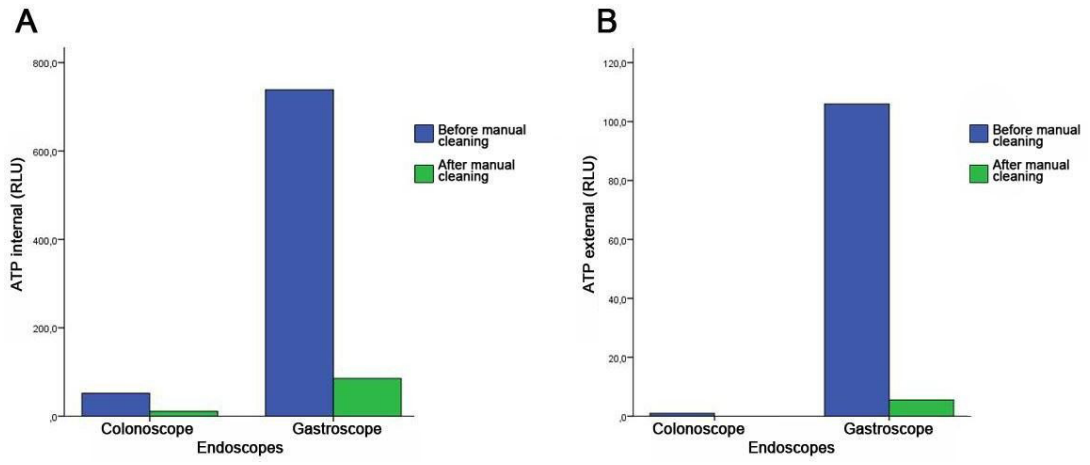

Figure 2. Comparison of ATP results of gastroscopes and colonoscopes tested before and after manual cleaning internally (A) and externally (B).

After manual cleaning, the internal part of the both endoscopes types were more contaminated with biological soil than the external part ($p<0.001$). Overall, 56.5% of endoscopes had ATP levels greater than 200 RLU before manual cleaning and 8% after cleaning.

For determining bacterial contamination levels, endoscopes flushes were tested by a combination of microbial culture and qPCR for total bacterial load. Microbial culture analysis demonstrated that 33% of the endoscopes tested grew bacteria before manual cleaning and 11% grew bacteria after manual cleaning. The maximum number of culturable bacteria prior to manual cleaning was 4 log10 and 2 log10 /mL after manual cleaning.

Isolated microorganisms were principally *Escherichia coli* (39%), *Staphylococcus coagulase negative* (19%) and *Klebsiella* spp. (17%).

For the working endoscope channel bacterial load analysis, the median values from before and after manual cleaning is 6 log10 bacteria/mL (range 3 log10 to 7 log10 bacteria/mL). There was a significant reduction in total bacterial load as measured by qPCR after manual cleaning ($p=0.03$) (Figure 18). The total bacterial load of colonoscopes following manual cleaning was significantly higher than the total bacterial load on gastroscopes ($p<0.001$).

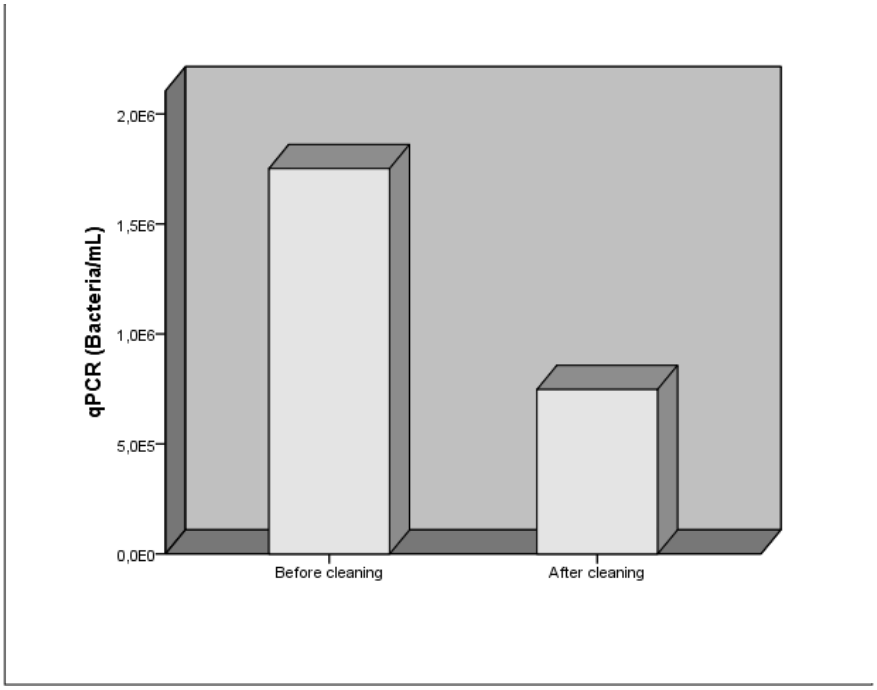

Figure 18. Comparison of qPCR results (Bacteria/mL) tested on clinically used gastrointestinal endoscopes before and after manual cleaning.

The correlation analysis among the variables cited above showed that ATP and qPCR values presented a positive and significant correlation ($p<0.001$ for colonoscopes and $p=0.035$ for gastroscopes).

4 I DISCUSSION

The efficacy of the reprocessing process of flexible gastrointestinal endoscopes was evaluated by measuring biological soil utilizing various ATP test kits and comparing ATP results to microbial load. There is frequent warning about the peculiarities of the physical structure of endoscopes that threaten reprocessing quality and safety. Therefore, a suitable bedside test to determine if an endoscope is clean prior to disinfection is a necessary quality control tool.

Accomplishing endoscope reprocessing recommendations involves a careful practice based on the range of procedures, equipment and potential risks involved in the process. Failures of endoscope reprocessing can result in contaminated endoscopes being used on patients and result in infection and outbreaks of HAI (KOLA, et al. 2015; PETERSEN, et al. 2017). This reality justifies the arsenal produced at the area, such as, disinfection agents, automated equipment, guidelines and reprocessing quality control aimed at improving patient safety (BISSET, et al. 2006; GASTMEIER; VONBERG, 2014; CDC, 2017). Endoscope reprocessing failures can be related to human performance, inadequate materials and equipment with structural and maintenance problems; and also organizational and environmental problems (WEBER; RUTALA, 2013; GAMBLE; DUCKWORTH; RIDGWAY, 2007;

LANGLAY, 2013).

Even though endoscope reprocessing evaluation may detect possible failures and to avoid patient contamination, some recommendations are controversial among international guidelines. Microbial culture is being used for detecting failures of endoscope reprocessing and endoscope damage, during endoscope contamination investigation (SAVIUC, et al. 2015; MUSCARELLA, et al. 2010) but is also used in clinical practice for quality control. Nevertheless, some guidelines do not include surveillance cultures as an endoscope reprocessing quality control given the time necessary for results, cost and difficulties in interpreting results, especially regarding environmental microorganism isolation (PETERSEN, et al. 2017; AORN, 2017).

Considering that endoscope reprocessing is a multistep process and that cleaning is considered crucial for disinfection success; monitoring cleaning procedures can be effective on endoscope reprocessing quality control (ALFA; FATIMA; OLSON, 2013). Currently, visual inspection is recommended as gastrointestinal endoscope manual cleaning evaluation procedure (CDC, 2017).

However, visual inspection recommendation has limitations, such as the subjectivity inherent to the qualitative evaluation process without pre-established criteria; the black color of the outside of the endoscopes that makes detecting soil difficult; and the presence of narrow internal channels in the endoscopes that make it impossible to visualize the endoscope internal surfasse (VISRODIA, et al. 2014). We have found that gastrointestinal endoscope internal surfaces were contaminated with higher amounts of biological soil than the external part of the endoscope. Visual inspection obviously is inadequate for detecting soil contaminating internal channels.

We measured endoscope contamination utilising ATP, qPCR and culture before and after manual cleaning. ATP test has been refereed as method to evaluate endoscope manual cleaning efficiency (PAROHL, et al. 2017; FUSHIMI, et al. 2013). ATP is present in all living organisms as an energy source, and in commercial test kits its presence is detected by the reaction of ATP with luciferase which converts ATP into visible light (SHAMA; MALIK, 2013). The use of ATP as an indicator of biological soil on test surfaces, including gastrointestinal endoscopes, has the advantage of being rapid with testing time taking less than 5 minutes (SETHI, et al. 2017).

However, some possible limitations on the use of ATP as a cleaning test monitoring are presented in the literature, such as, low accuracy and reproducibility among commercially available brands99 different brands of ATP luminometer read on different RLU scales so comparison between brands is difficult and limited sensitivity in detecting low levels of ATP (WHITELY, et al. 2015). Thus, it is essential to use in clinical practice an institutional protocol with recommendations for interpretation of the results obtained with each type/brand of ATP test adopted.

The mean ATP values assessed on internal channels of gastrointestinal

endoscopes tested prior to manual cleaning were 738 RLU in gastroscopes and 52 RLU in colonoscopes. After cleaning, these values decreased significantly to 85 and 11 RLU, respectively. Similar study (ALFA; FATIMA; OLSON, 2013), using different brand, demonstrated a reduction from 1315 RLU in the biopsy channels and 39.3 RLU in the colonoscopes air and water channels before cleaning to 20 RLU and 15.2 RLU, respectively, after cleaning.

Clinical studies evaluating gastrointestinal endoscopes cleaning procedure with the same ATP devices used in this study have not been found in the literature. However, comparison of three ATP test brands with in vitro contamination on metal surfaces demonstrated statistically significant difference before and after cleaning using Hygiena Ultrasnap device (SCIORTINO; GILES, 2012).

Although we haven't found significant correlation between the results of ATP tests and microbial culture, the correlation between biological soil (ATP) and total bacterial load, as determined by qPCR, was statistically significant. It should be noted that the majority of samples post cleaning had no microbial growth, this suggests that the pre-cleaning and manual cleaning of the endoscopes were effective in reducing their levels of contamination.

5 I CONCLUSION

Endoscope manual cleaning analysis demonstrated the efficiency in reducing biological soil ($p < 0.001$) and microbial contamination ($p = 0.03$), with a low percentage of the sample with viable microorganisms. This study confirms the importance on manual cleaning on endoscope reprocessing and its relation with microbial contamination, therefore, stating the importance of additional researches on endoscope reprocessing quality control for assuring a safe procedure.

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ÍNDICE REMISSIVO

A

Acidente de trânsito 177, 207, 208
Acidentes de Trânsito e mortalidade 198
Acidentes de transporte terrestre 198, 199, 206, 208
Ações integradas da saúde 151
Acolhimento 5, 6, 7, 8, 10, 15, 57, 93, 144, 147, 217, 221, 225, 227
Alérgenos 42, 43, 44, 45, 46, 47, 48, 49, 50, 51
Ambiente 42, 43, 45, 47, 48, 64, 92, 131, 145, 146, 210, 211, 215, 216, 217, 218, 219, 224, 226, 227, 230, 264, 277
Amputação 276, 279, 280, 281
Atenção básica 5, 6, 7, 8, 9, 10, 12, 14, 15, 50, 56, 62, 68, 73, 75, 76, 89, 122, 142, 151, 153, 154, 156, 157, 159, 161, 166, 173, 175, 212, 215, 224, 284
Atenção primária à saúde 2, 75, 160, 161, 162, 165, 169, 172
Autocuidado 54, 55, 59, 60, 82, 90, 95, 99, 239

B

Bioética 19, 25, 244, 286

C

Câncer 18, 19, 20, 22, 23, 24, 25, 26, 27, 30, 31, 32, 33, 34, 37, 38, 39, 40, 41, 67, 95, 99, 137, 152, 245, 246, 248, 249, 250, 252, 254, 255
Cicatrização 256, 260, 261, 263, 264, 267, 269, 273, 274, 275
Comunidade ribeirinha 210, 219
Controle de qualidade 101
Cuidado paliativo 234, 235, 242, 244

D

Dependência química 185, 191, 195
Determinantes sociais da saúde 16, 126, 127, 128, 132, 135
Diabetes melito 276, 278
Dispositivo de proteção da cabeça 177
Doenças das Vias Respiratórias 43
Doenças do sistema circulatório 126
Doenças raras 54, 61
Dor 9, 34, 36, 37, 39, 47, 60, 64, 65, 66, 67, 68, 70, 71, 72, 73, 74, 75, 76, 136, 142, 234, 235, 239, 241, 249, 251, 252, 267, 279

E

Educação permanente 5, 8, 10, 145, 147, 160
Efeitos colaterais 20, 30, 31, 32, 33, 36, 38, 39, 40, 156, 229
Endoscópios gastrointestinais 101
Enfermeiros 4, 73, 75, 91, 111, 142, 146, 147, 149, 156, 159, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 265
Epidemiologia 2, 89, 125, 134, 184, 207, 208
Estilo de vida 84, 85, 89, 117, 210, 211, 212, 215, 276
Estratégia de saúde da família 5, 14, 70, 74, 78, 85, 87, 90, 116, 125, 163, 165, 173, 174, 175

F

Fatores de risco 46, 47, 62, 77, 78, 79, 80, 82, 83, 84, 85, 86, 87, 88, 89, 90, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 124, 125, 127, 134, 142, 178, 193, 196, 197, 208, 274, 275, 278, 279, 286
Ferimentos 98, 276

G

Gestão em saúde 91, 174

H

Hiperdia 77, 78, 79, 80, 81, 82, 83, 84, 85, 87, 88, 89, 90, 116, 117, 125, 284
Hospital 20, 23, 30, 31, 33, 35, 36, 41, 43, 44, 64, 75, 91, 92, 93, 95, 96, 97, 98, 99, 103, 110, 111, 116, 125, 143, 144, 146, 161, 178, 180, 187, 208, 229, 234, 243, 245, 248, 249, 250, 256, 257, 258, 261, 266, 270, 275, 288
Humanização 6, 7, 10, 73, 144, 148, 149, 171, 241

I

Incidência 2, 38, 40, 41, 64, 68, 70, 71, 74, 79, 80, 84, 95, 112, 113, 119, 120, 122, 153, 154, 178, 207, 238, 254, 284
Insuficiência renal crônica 112, 113, 114, 117, 118, 119, 120, 121, 123, 124, 125
Intensidade 46, 64, 66, 71, 72, 118, 199, 240, 261, 263, 265, 274

L

Laser 261, 262, 263, 264, 265, 266, 267, 268, 270, 273, 274, 275
Lesão autoprovocada 136, 137, 138, 139, 140, 141
Lesão por pressão 256, 258, 261, 270, 271, 275
Lesões 65, 67, 86, 87, 88, 121, 137, 176, 177, 178, 179, 181, 182, 183, 184, 186, 198, 199, 203, 256, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 269, 276, 280, 281, 282, 283
Leucemia 30, 31, 32, 33, 35, 36, 38, 40, 41

M

Médicos 23, 64, 72, 73, 99, 159, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175
Meio ambiente 47, 210, 211, 215, 216, 218, 219, 277
Morbidade 83, 130, 134, 135, 177
Mortalidade 31, 80, 83, 123, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 141, 143, 153, 198, 199, 200, 201, 202, 203, 205, 206, 207, 208, 280
Motocicleta 176, 177, 178, 180, 181, 182, 183, 203

P

Pacientes internados 91, 93, 94, 95, 96, 97, 99, 116, 117, 125, 150, 233, 235, 236, 237, 275
Perfil de saúde 91, 235, 236
Política 6, 7, 10, 11, 13, 14, 15, 16, 22, 28, 61, 129, 133, 164, 173, 196, 212, 214, 215, 218, 219, 222, 223, 231, 246, 254, 284
Prática profissional 163, 225
Prevalência 42, 43, 47, 48, 49, 50, 68, 70, 71, 75, 78, 79, 82, 83, 85, 86, 89, 90, 113, 115, 116, 117, 118, 119, 121, 122, 123, 124, 125, 127, 143, 182, 183, 186, 194, 195, 197, 198, 214, 215, 238, 240, 274, 279
Processo de trabalho 10, 93, 144, 149, 151, 160
Promoção em saúde 234

Q

Qualidade de vida 14, 24, 30, 31, 32, 33, 34, 41, 43, 54, 55, 56, 59, 60, 61, 63, 79, 83, 85, 87, 90, 134, 188, 210, 212, 214, 233, 234, 245, 246, 249, 250, 251, 252, 276, 277, 280, 281, 283, 286
Quimioterapia 26, 30, 31, 32, 33, 36, 38, 39, 40, 41

R

Reforma psiquiátrica 196, 221, 224, 225, 232
Ribeirinhos 210, 211, 214, 215, 216, 217, 218, 219

S

Saúde ambiental 210
Saúde do homem 100, 129, 133, 245, 246, 247, 252, 253, 254, 284
Saúde mental 142, 143, 185, 186, 187, 188, 196, 197, 221, 223, 224, 225, 227, 228, 232
Saúde pública 1, 2, 4, 7, 11, 13, 16, 23, 28, 37, 43, 45, 48, 52, 62, 74, 75, 78, 79, 86, 112, 113, 134, 135, 142, 143, 151, 161, 174, 178, 183, 184, 186, 196, 197, 198, 199, 200, 202, 207, 208, 210, 231, 232, 235, 243, 244, 278, 288
Serviços comunitários de saúde mental 185
Síndrome de guillain-barré 256, 257
Sistema de registro 151, 153
Software 68, 103, 127, 151, 155, 238, 261, 262, 269
Suicídio 136, 137, 139, 140, 141, 142, 143, 191, 195, 196

T

Tentativa de suicídio 136, 191
Terapia comunitária 221, 223, 224, 225, 226, 231, 232
Terapias complementares 27, 221
Trifosfato de adenosina 101
Tuberculose 1, 2, 3, 4, 151, 152, 154, 160, 161, 162
Tuberculose na atenção básica 151, 161

U

Unidade de terapia intensiva 144, 145, 146, 147, 148, 150, 257, 261

V

Vulnerabilidade em saúde 18