AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Estimation and evaluation of Minimum Inhibitory Concentration of Meropenem by E-Strip Method on Hospital Acquired Pathogen

Shweta Sao[1]*, Arijeet Mukherjee[1]
1. Department of Life Sciences, Dr. C.V. Raman University Kargi road Kota Bilaspur (C.G.) 495013.

ABSTRACT

Hospitals are always acted as a source of infection to patients admitted to them. The terms hospital infection, hospital-acquired infection or nosocomial infection are applied to infections developing in hospitalized patients, not present or in incubation at the time of their admission. Such infection may become evident during their stay in hospital or, sometimes, only after their discharge. Approximately 5-10 % of patients admitted to acute care hospitals in developed countries, and more than 25% of such patients in developing countries, have been found to acquire infections which were not present or incubating at the time of admission. Such hospital-acquired, or nosocomial infections add to the morbidity, mortality, and costs that one might expect from the underlining illness alone. To fight these infections hospital personals employs many techniques and treatments to minimize the risk of certain infections. One of them is the use of antibiotics (like Meropenem) to prevent or control the spreading of such infections. Meropenem is an ultra-broad spectrum injectable antibiotic used to treat a wide variety of infections. It is a beta-lactam and belongs to the subgroup of carbapenem, similar to imipenem and ertapenem. Most hospital acquired pathogens show both sensitive and resistant results for Meropenem. So it is necessary to evaluate the minimum inhibitory concentration (MIC) of Meropenem which would help the doctors to treat the patients infected by hospital acquired pathogens in a distinctive manner. So for the evaluation of MIC of Meropenem we are using the E-Strip Method which is useful for quantitative determination of susceptibility of bacteria to antibacterial agents. The system comprises of a predefined quantitative gradient which is used to determine the Minimum Inhibitory Concentration (MIC) in mcg/ml of different antimicrobial agents against microorganisms as tested on appropriate agar media, following overnight incubation. Thus by this experiment we will be able to provide satisfactory data to the hospital personals for the treatment of hospital acquired infections using Meropenem.

Keywords: Minimum Inhibitory Concentration, Meropenem, E-Strip Method.

*Corresponding Author Email: drshwetasao02@gmail.com
Received 17 June 2015, Accepted 04 July 2015

Please cite this article as: Sao S *et al.*, Estimation and evaluation of Minimum Inhibitory Concentration of Meropenem by E-Strip Method on Hospital Acquired Pathogen. American Journal of PharmTech Research 2015.

INTRODUCTION

A hospital is a health care institution providing patient treatment by specialized staff and equipment. Hospitals are usually funded by the public sector, by health organizations (for profit or nonprofit), health insurance companies, or charities, including direct charitable donations. Historically, hospitals were often founded and funded by religious orders or charitable individuals and leaders. Today, hospitals are largely staffed by professional physicians, surgeons, and nurses, whereas in the past, this work was usually performed by the founding religious orders or by volunteers. However, there are various Catholic religious orders, such as the Alexians and the Bon Secours Sisters, which still focus on hospital ministry today. There are over 17,000 hospitals in the world. Some patients go to a hospital just for diagnosis, treatment, or therapy and then leave ('outpatients') without staying overnight; while others are 'admitted' and stay overnight or for several days or weeks or months ('inpatients'). Hospitals usually are distinguished from other types of medical facilities by their ability to admit and care for inpatients whilst the others often are described as clinics. Approximately 5-10 % of patients admitted to acute care hospitals in developed countries, and more than 25% of such patients in developing countries, have been found to acquire infections which were not present or incubating at the time of admission; this infections are termed as Hospital Acquired Infection (HAI) or Nosocomial Infection. A nosocomial infection, also known as a hospital-acquired infection or HAI, is an infection whose development is favored by a hospital environment, such as one acquired by a patient during a hospital visit or one developing among hospital staff. Such infections include fungal and bacterial infections and are aggravated by the reduced resistance of individual patients.

Factors predisposing to hospital infection

1. Pre-existing condition.

2. Need for invasive devices (IV, U, ET).

3. Effect of surgery (skin wound, tissue trauma, opening colonized viscous, immobilization, implants of foreign material (joint prostheses, arterial graft).

4. Effect of antibiotic treatment (colonization by resistant bacteria and fungi).

5. Effect of immunosuppressive treatment (corticosteroids, cancer chemotherapy, radiotherapy, transplant immune supression).

6. Exposure to health care workers and other patients who may transmit pathogens.

7. Exposure to pathogens in the hospital environment.

Common types of Nosocomial Infection

- Hospital-acquired Urinary Tract Infection.
- Hospital-acquired Bacteremia.
- Hospital-acquired Pneumonia.
- Hospital-acquired Infections due to the Hepatitis B and Hepatitis C viruses.
- Hospital-acquired Tetanus.
- Hospital-acquired Acute Gastroenteritis.
- Hospital-acquired Wound Infection.
- Self-Infection (Auto-Infection).
- Cross-Infection.

Known nosocomial infections

- Ventilator-associated pneumonia
- *Staphylococcus aureus*.
- Methicillin resistant *Staphylococcus aureus*
- *Candida abacas*
- *Pseudomonas aeruginosa*
- *Acinetobacter baumannii*
- *Stenotrophomonas maltophilia*
- *Clostridium difficile*
- Tuberculosis
- Urinary tract infection
- Hospital-acquired pneumonia
- Gastroenteritis
- Vancomycin-resistant *Enterococcus*
- Legionnaires' disease

Nosocomial infections are commonly transmitted when hospital officials become complacent and personnel do not practice correct hygiene regularly. Also, increased use of outpatient treatment in recent decades means that a greater percentage of people who are hospitalized today are likely to be seriously ill with more weakened immune systems than in the past. Moreover, some medical procedures bypass the body's natural protective barriers. Since medical staff moves from patient to patient, the staff themselves serves as a means for spreading pathogens. Essentially, the staff acts as vectors.

Route of transmission

The basic sources are the common flora of the patients, hospital personals, contaminated hospital environment, medical instruments etc. Except these sources some are described below Table 1 which is very crucial for the knowledge of nosocomial infection: Contact transmission is divided into two subgroups: direct-contact transmission and indirect-contact transmission (Table 2)

Table 1 Sources for nosocomial infection

Main routes of transmission	
Route	**Description**
Contact transmission	The most important and frequent mode of transmission of nosocomial infections is by direct contact.
Droplet transmission	Transmission occurs when droplets containing microbes from the infected person are propelled a short distance through the air and deposited on the host's body; droplets are generated from the source person mainly by coughing, sneezing, and talking, and during the performance of certain procedures, such as bronchoscopy.
Airborne transmission	Dissemination can be either airborne droplet nuclei (small-particle residue {5 μm or smaller in size} of evaporated droplets containing microorganisms that remain suspended in the air for long periods of time) or dust particles containing the infectious agent. Microorganisms carried in this manner can be dispersed widely by air currents and may become inhaled by a susceptible host within the same room or over a longer distance from the source patient, depending on environmental factors; therefore, special air-handling and ventilation are required to prevent airborne transmission. Microorganisms transmitted by airborne transmission include *Legionella*, *Mycobacterium tuberculosis* and the rubeola and varicella viruses.
Common vehicle transmission	This applies to microorganisms transmitted to the host by contaminated items, such as food, water, medications, devices, and equipment.
Vector borne transmission	This occurs when vectors such as mosquitoes, flies, rats, and other vermin transmit microorganisms.

Table 2: Routes of contact transmission

Routes of contact transmission	
Route	**Description**
Direct-contact transmission	This involves a direct body surface-to-body surface contact and physical transfer of microorganisms between a susceptible host and an infected or colonized person, such as when a person turns a patient, gives a patient a bath, or performs other patient-care activities that require direct personal contact. Direct-contact transmission also can occur between two patients, with one serving as the source of the infectious microorganisms and the other as a susceptible host.
Indirect-contact transmission	This involves contact of a susceptible host with a contaminated intermediate object, usually inanimate, such as contaminated instruments, needles, or dressings, or contaminated gloves that are not changed between patients. In addition, the improper use of saline flush syringes, vials, and bags has been implicated in disease transmission in the US, even when healthcare workers had access to gloves, disposable needles, intravenous devices, and flushes.[20]

Table 3: Representation of MIC Obtained from Different Sample Plate

S. No.	Sample Name	Organism Identified	Antibiotic used	Incubation Period	Result	MIC Values of Plates mcg/ml
1	U-1	*Kleb. pneumoniae*	Meropenem	18 to 24 hrs	RES	-
2	U-2	*P. aeruginosa*	Meropenem	18 to 24 hrs	RES	-
3	U-3	*E. coli*	Meropenem	18 to 24 hrs	SEN	125
4	U-4	*E. coli*	Meropenem	18 to 24 hrs	SEN	0.94
5	U-5	*Kleb. pneumoniae*	Meropenem	18 to 24 hrs	SEN	19
6	B-1	*Staph. Aureus*	Meropenem	18 to 24 hrs	SEN	38
7	B-2	*C. diversus*	Meropenem	18 to 24 hrs	RES	-
8	B-6	*MRSA*	Meropenem	18 to 24 hrs	RES	-
9	SP-1	*P. species*	Meropenem	18 to 24 hrs	INTR	.25
10	SP-2	*C. diversus*	Meropenem	18 to 24 hrs	RES	-
11	SP-3	*P. aeruginosa*	Meropenem	18 to 24 hrs	RES	-
12	SP-4	*Kleb. Pneumoniae*	Meropenem	18 to 24 hrs	SEN	.50
13	M-1	*Kleb. Pneumoniae*	Meropenem	18 to 24 hrs	SEN	2
14	M-3	*Kleb. pneumoniae*	Meropenem	18 to 24 hrs	RES	-
15	M-4	*P. aeruginosa*	Meropenem	18 to 24 hrs	RES	-
16	M-5	*P. aeruginosa*	Meropenem	18 to 24 hrs	SEN	6
17	ST-1	*E. coli*	Meropenem	18 to 24 hrs	SEN	0.087
18	ST-2	*E. coli*	Meropenem	18 to 24 hrs	SEN	19
19	ST-3	*Kleb. pneumoniae*	Meropenem	18 to 24 hrs	RES	-
20	ST-4	*P. aeruginosa*	Meropenem	18 to 24 hrs	INTR	37
21	ST-5	*Kleb. pneumoniae*	Meropenem	18 to 24 hrs	RES	-

Note: For Comparing the Result the CLSI Guideline is provided in Material and Method Section.

Diagnosis and Prevention

Hospitals have sanitation protocols regarding uniforms, equipment sterilization, washing, and other preventive measures. Thorough hand washing and/or use of alcohol rubs by all medical personnel before and after each patient contact is one of the most effective ways to combat nosocomial infections. More careful use of antimicrobial agents, such as antibiotics, is also considered vital.

Antimicrobial Susceptibility Testing

The visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A lower MIC is an indication of a better antimicrobial agent. A MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism.

Determination:

MICs can be determined by agar or broth dilution methods usually following the guidelines of a reference body such as the CLSI, BSAC or EUCAST. There are several commercial methods available, including the well established E-test strips and the recently launched Oxoid MICE

valuator method. The E-test system comprises a predefined and continuous concentration gradient of different antimicrobial agents, which when applied to inoculated agar plates and incubated, create ellipses of microbial inhibition. The MIC is determined where the ellipse of inhibition intersects the strip, and is easily read off the MIC reading scale on the strip.

Clinical significance:

Clinically, the minimum inhibitory concentrations are used not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents. Currently, there are a few web-based, freely accessible MIC databases.

Carbapenem:

Carbapenems (Figure 1) are a class of β-lactam antibiotics with a broad spectrum of antibacterial activity. They have a structure that renders them highly resistant to most β-lactamases. Carbapenem antibiotics were originally developed from thienamycin, a naturally derived product of *Streptomyces cattleya* Carbapenems are one of the antibiotics of last resort for many bacterial infections, such as *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae*. Recently, alarm has been raised over the spread of drug resistance to carbapenem antibiotics among these coliforms, due to production of the New Delhi metallo-β-lactamase, NDM-1. There are currently no new antibiotics in the pipeline to combat bacteria resistant to Carbapenems, and worldwide spread of the resistance gene is considered a potential nightmare scenario.

Figure 1: Streptomyces cattleya

Meropenem

Meropenem **(**Figure 2) is an ultra-broad spectrum injectable antibiotic used to treat a wide variety of infections. It is a beta-lactam and belongs to the subgroup of carbapenem, similar to imipenem and ertapenem. Meropenem was originally developed by MJ Pharmaceuticals. It is marketed in India by Nirlife and Positive Medicare with the brand name Meronir and Carnem respectively and outside India by AstraZeneca with the brand names Monan and Meronem. It gained US FDA

approval in July 1996. It penetrates well into many tissues and body fluids including the cerebrospinal fluid, bile, heart valves, lung, and peritoneal fluid.

Figure 2: Meropenem

Mechanism of action

Meropenem is bacteriocidal except against Listeria monocytogenes where it is bacteriostatic. It inhibits bacterial wall synthesis like other beta-lactamantibiotics. In contrast to other beta-lactams, it is highly resistant to degradation by beta-lactamases or cephalosporinases. Resistance generally arises due to mutations in penicillin binding proteins, production of metallo-beta-lactamases, or resistance to diffusion across the bacterial outer membrane. Unlike imipenem, it is stable to dehydropeptidase-1and can therefore be given without cilastatin.

Indications

The spectrum of action includes many gram-positive and negative bacteria (including Pseudomonas) and anaerobic bacteria. The overall spectrum is similar to imipenem although meropenem is more active against Enterobacteriaceae and less active against gram-positive bacteria. It is also very resistant to extended-spectrum beta lactamases but may be more susceptible to metallo-beta-lactamases. Meropenem is frequently given in the treatment of febrile neutropenia. This condition frequently occurs in patients with hematological malignancies and cancer patients receiving anticancer drugs that cause bone marrow suppression.

Presence of Meropenem resistant Hospital Acquired Pathogen:

Meropenem Resistant *Pseudomonas aeruginosa.*

Meropenem Resistant *Klebsiella*

Meropenem Resistant *Acinetobacter*.

Meropenem Resistant Extended spectrum beta lactamase.

Meropenem Resistant *Enterobactor.*

Meropenem Resistant *Pneumococcus.*

Determination of Minimum Inhibitory Concentration by E-Strip Test

The Epsilometer test (usually abbreviated E-strip test) is a laboratory test used by microbiologists to determine whether or not a specific strain of bacterium or fungus is susceptible to the action of a specific antibiotic. This is most commonly used in the setting of medicine, where a particular organism has been found to infect a patient, and the doctor treating the patient is seeking guidance on what concentration of antibiotic is suitable.

History

The principle of the epsilometer test was first described in 1988 and was introduced commercially in 1991 by AB Biodisk.

Principle

The E-strip test is basically an agar diffusion method. The E-strip test utilizes a rectangular strip that has been impregnated with the drug to be studied. A lawn of bacteria is spread and grown on an agar plate, and the E-strip test strip is laid on top; the drug diffuses out into the agar, producing an exponential gradient of the drug to be tested. There is an exponential scale printed on the strip. After 24 hours of incubation, an elliptical zone of inhibition is produced and the point at which the ellipse meets the strip gives a reading for the minimum inhibitory concentration (MIC) of the drug.

Validation

The test has been validated for many organisms against the broth/agar dilution method and shown to have excellent correlation. This is a partial list of organisms and antibiotics for which the test has been validated.

Importance and objective of the work

Nosocomial infections are commonly transmitted when hospital officials become complacent and personnel do not practice correct hygiene regularly. Also, increased use of outpatient treatment in recent decades means that a greater percentage of people who are hospitalized today are likely to be seriously ill with more weakened immune systems than in the past. Moreover, some medical procedures bypass the body's natural protective barriers. Since medical staff moves from patient to patient, the staff themselves serves as a means for spreading pathogens. Essentially, the staff acts as vectors. So it is our duty as a microbiologist to implant certain ideas that will help to control such infections and fill the future with happiness. Many Hospital Acquired Pathogens give intermediate result when treated with Meropenem. So it is necessary evaluate the Minimum Inhibitory Concentration of meropenem for those particular pathogens, so that doctors could treat their patients in a better way. So for the evaluation of MIC of Meropenem we are using the E-Strip Method which is useful for quantitative determination of susceptibility of bacteria to antibacterial agents.

MATERIALS AND METHOD

Prepare the medium of choice from dehydrated powder according to the directions specified on the label. Cool the sterilized molten medium to 45-50°C and pour in sterile, dry Petri plates on a leveled surface, to a depth of 4 ± 0.2 mm and allow to solidify. Few droplets appearing on the surface of the medium following cooling do not matter. Hence, once poured, Petri plates containing media should not be dried on laminar flow and can be used immediately for swabbing.

Preparation of Inoculums

Use only pure cultures. Confirm by Gram-staining before starting susceptibility test. Transfer 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08 - 0.13 OD turbid suspension at 620 nm). Also direct colony suspension method can be used. Prepare a direct colony suspension, from 18-24 hour old non-selective media agar plate in broth or saline. Adjust the turbidity to that of standard 0.5 McFarland. This method is recommended for testing fastidious organisms like *Haemophilus* spp., *Neisseria* spp, Streptococci and for testing Staphylococci for potential Methicillin or Oxacillin resistance.

Test Procedure

1. Prepare plates with suitable make of Mueller Hinton Agar for rapidly growing aerobic organisms as mentioned above. For fastidious organisms such as Streptococci, Mueller Hinton Agar is supplemented with 5% sterile, defibrinated blood. For *Haemophilus* spp, Haemophilus Test Agar Base (M1259) with added supplement (FD117) is to be used.

2. Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking.

3. Remove MIC strip container from cold and keep it at room temperature for 15 minutes before opening.

4. Remove one applicator from the self sealing bag stored at room temperature.

5. Hold the applicator in the middle and gently press its broader sticky side on the centre of MIC strip.

6. Lift the applicator along with attached MIC strip.

7. Place the strip at a desired position on agar plate swabbed with test culture. Gently turn the applicator clockwise with fingers. With this action, the applicator will detach from the strip.

8. DO NOT PRESS MIC STRIP. Within 60 seconds, MIC strip will be adsorbed and will firmly adhere to the agar surface.

9. MIC strip should not be repositioned or adjusted once placed.

10. Transfer plates in the incubator under appropriate conditions.

RESULTS AND DISCUSSION

After an incubation period of 18 to 24 hrs, Sensitive, Intermediate and Resistant zones have appeared in which Meropenem E-Strip was placed. These plates are as follows:

Plate: U-1 (figure 3) **MIC**: Fully Resistant **Organism:** *Klebsiella pneumoniae.*

Plate: U-2 (Figure 4) **MIC**: Fully Resistant **Organism:** *Pseudomonas aeruginosa.*

Plate: U-3 (Figure 5) **MIC**: Sensitive **Organism**: *Escherichia coli.*

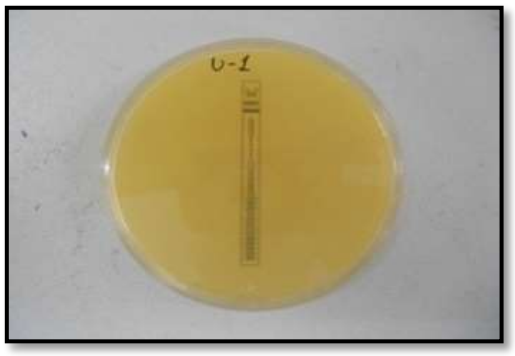

Figure 3: Klebsiella pneumoniae

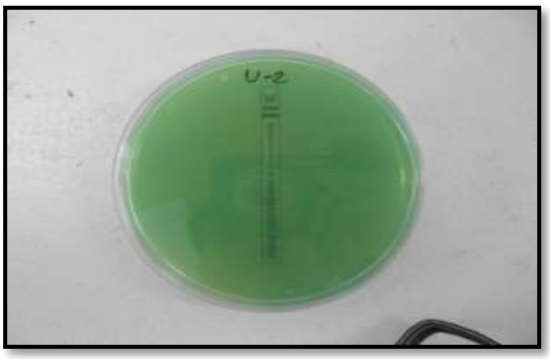

Figure 4: Pseudomonas aeruginosa

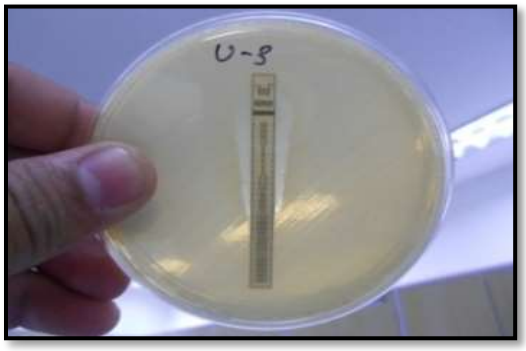

Figure 5: Escherichia coli

Hospital-acquired bacterial infections may dominate the headlines, but most infections occur in the community. Indeed, 80% of the antibiotic prescribing takes place in the community – in local practices, daycare centers and long-term care facilities such as nursing homes and rehabilitation centers. Most patients hospitalized in the Intensive Care Units after being discharged continue to carry Extended Spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* over prolonged periods. Continued carriage of such strains may contribute to their extra hospital propagation.

Some patients go to a hospital just for diagnosis, treatment, or therapy and then leave ('outpatients') without staying overnight; while others are 'admitted' and stay overnight or for several days or weeks or months ('inpatients'). Hospitals usually are distinguished from other types of medical facilities by their ability to admit and care for inpatients whilst the others often are described as clinics. Approximately 5-10 % of patients admitted to acute care hospitals in developed countries, and more than 25% of such patients in developing countries, have been found to acquire infections which were not present or incubating at the time of admission; these infections are termed as Hospital Acquired Infection (HAI) or Nosocomial Infection. Nosocomial infections are commonly transmitted when hospital officials become complacent and personnel do not practice correct hygiene regularly. Also, increased use of outpatient treatment in recent decades means that a greater percentage of people who are hospitalized today are likely to be seriously ill with more weakened immune systems than in the past. Moreover, some medical procedures bypass the body's natural protective barriers. Since medical staff moves from patient to patient, the staff themselves serves as a means for spreading pathogens. Essentially, the staff acts as vectors. So it is our duty as a microbiologist to implant certain ideas that will help to control such infections and fill the future with happiness.

REFERENCE

1. Abrutyn E, Goldmann D, Scheckler W, eds. Saunders infection control reference service (2nd ed). Philadelphia, Saunders, 2001
2. Baccan, N.; Andrade, J. C.; Godinho, O. E. S.; Barone, J. S. Química analítica quantitativa elementar. São Paulo: Edgard Blücher.
3. Bennett JV and Brachman PS, eds. Hospital infections(4th ed). Philadelphia, Lippincott-Raven, 1998.
4. Diaz, R.B.; Vessoni Penna, T.C.; Takeuti, C.E.; Taira, E.T.; Guimarães, T. Centralização do processo de desinfecção de materiais semi-críticos e não-críticos no Hospital Santa Cruz: melhoria da qualidade e redução de custos. Laes & Hae, 2000;6:68-78.
5. Glynn A et al. Hospital-acquired infection: Surveillance, policies and practice. London, Public Health Laboratory
6. Hazard Analysis Critical Control Point Evaluation. A guide to identifying hazards and assessing risks associated with food preparation and storage, Bryan FL, 1992
7. Kessler M, Huu TC, Mariot A, Chanliau, J. Hemodialysis associated complications due to sterilizing agents ethylene oxide and formaldehyde. Contrib. Nephrol 1988;62:13-23.

8. Mayhall C Glen, ed. Hospital epidemiology and infection control (2nd ed). Philadelphia, Lippincott, Williams & Wikilins 1999
9. Mcdonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin. Microbiol. Rev 1999;12:147-179.
10. Mazzola, PG, Vessoni Penna TC, Machoshvili IA, Rogrigues RC. Eficácia dos Agentes Químicos no Programa de Limpeza, Desinfecção e Esterilização. Laes & Haes, v.127, p.100-126, 2000.
11. Rutala WA. APIC guidelines for selection and use of disinfectants. Am. J. Infect. Control.
12. Rutala WA. Disinfection, sterilization and antiseptics in health care. New York: Polyscience Publishers
13. Rutala WA. Selection and use of disinfections in health care. In: __. Hospital epidemiology and infection control. Philadelphia: Mayhall
14. Sagripanti JL, Bonifacino, A. Effects of salt and serum on the sporocidal activity of liquid disinfectants. J. AOAC Intern.
15. Traumann, MM, Michalsky T, Wiedeck H, Radosavljevic V, Ruhnke, M. Tap Water Colonization with Pseudomonas aeruginosa in a surgical intensive care unit (ICU) and relation to Pseudomonas infections of ICU patients. Infect. Control Hosp. Epidemiol
16. Traumann, MM, Michalsky T, Wiedeck H, Radosavljevic V, Ruhnke, M. Tap Water Colonization with Pseudomonas aeruginosa in a surgical intensive care unit (ICU) and relation to Pseudomonas infections of ICU patients. Infect. Control Hosp. Epidemiol 2001; 22:49-52, 2001.
17. Vessoni Penna, TC, Schaffner D, Abe LE, Machoshvili IA. Inactivation of Brazilian wild type and enterotoxigenic Escherichia coli by chlorine. J. Ind. Microbiol 1996;15: 57-61