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Editorial

Contents

From the very beginning, Journal of Hygiene Sciences is primarily focused on, acquiring the latest information about advancements in bio-technology of Disinfection and Microbiology, in the clinical as well as industrial segment and sincerely delivering it to our readers. Following this trend, another issue is brought up, which will share a vast range of topics and acquaint our readers with the latest trends and events in Disinfection and Microbiology.

Beginning with Antimicrobial Susceptibility Testing Agents, the Concept & Framework behind this is explained. Microorganisms isolated from patients are analyzed for Antimicrobial susceptibility testing in microbiology lab. The goal is to predict the in vivo success or failure of antibiotic therapy. The tests are performed under standardized conditions so that the results are reproducible. The test results should be used to guide antibiotic choice. The results of antimicrobial susceptibility testing should be combined with clinical information and experience when selecting the most appropriate antibiotic for the patient.

Current Trend deals with the Importance of Pre-cleaning and Disinfection of Surgical Instruments. To understand why it is important to Pre-clean and Disinfect the Surgical Instruments, it is necessary to know the past incidents that occurred when this criterion was neglected and the problems encountered. Also, learn their sources and the different ways by which these microorganisms have survived the disinfection procedure and caused infection. The latest trends in Pre-cleaning and Disinfection of Surgical Instruments will be discussed in the next issue.

In Profile segment familiarize with John Franklin Enders, from USA, who won Nobel Prize in Physiology or Medicine for the discovery of the ability of poliomyelitis viruses to grow in cultures of various types of tissue.

Nocardia species will be discussed in the Bug of the month segment, which are saprophytes and are present in soil water, or decaying organic. It forms partially acid-fast beaded branching filaments (acting as fungi, but being truly bacteria). The genus contains at least 25 species, of which 13 are pathogenic (Nocardiosis) to humans to both immune competent and immune compromised individuals.

Did You Know emphasizes on Antifungal agent which is a substance that kills fungi or inhibits its growth. There are many types of antifungal agents used to treat a range of illnesses and conditions caused by fungi. Some of these agents are topical while others are meant to be ingested. Many antifungal agents can be purchased over the counter for use without a doctor's guidance, but some are available only by prescription.

Best Practices segment will instruct the various modes of Microbiological testing in food industries. Microbiological examination of foods and food ingredients helps to assess safety to consumers, stability or shelf life under normal storage conditions, and the level of sanitation used during processing. Thus, routine examination of foods to detect selected pathogens is necessary. Most analyses look for indicator organisms, which are more rapidly enumerated. But in Rapid pathogen testing methods bacteria may be identified by many attributes, including fatty acid and carbon oxidation profiles. Miniaturized biochemical identification kits are becoming even more user-friendly, as many are now automated.

JHS team thanks all our readers for their support and contribution in making this journal a success. Feedback and suggestions are always invited.

Antimicrobial Susceptibility Testing Agents: Concept & Framework

Microorganisms isolated from patients are analysed for Antimicrobial susceptibility testing in microbiology lab. The goal of antimicrobial susceptibility testing is to predict the in vivo success or failure of antibiotic therapy. Tests are performed in vitro, and measure the growth response of an isolated organism to a particular drug or drugs. The tests are performed under standardized conditions so that the results are reproducible. The test results should be used to guide antibiotic choice. The results of antimicrobial susceptibility testing should be combined with clinical information and experience when selecting the most appropriate antibiotic for your patient.

Mechanisms of bacterial resistance are complex and not completely understood. Likewise, antimicrobial susceptibility testing has become more challenging with the continued emergence of unique resistance mechanisms. The goal of the microbiology laboratory in antibiotic susceptibility testing is to provide standardized in vitro susceptibility tests that can be reproduced from day to day and from laboratory to laboratory. Without reproducibility there is no scientific basis for therapy. Standardized guidelines for susceptibility testing are published and updated annually by the Clinical Laboratory Standards Institute (CLSI). These guidelines provide susceptibility testing methods that have been validated as accurate, reproducible, clinically relevant and predictive of clinical efficacy based on Pharmacokinetic* and outcome data. The raw data are either in the form of a zone size or Minimal Inhibitory Concentration(MIC). CLSI has published interpretation criteria for these tests, which has come from extensive testing and clinical correlation. CLSI guidelines recommend interpreting and reporting bacterial susceptibility results as susceptible, intermediate, or resistant (S, I, or R).

Susceptible: The "susceptible" category implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used for the site of infection. (CLSI definition)
Note that this definition says nothing about the chances of clinical success; in fact predicting clinical outcome based on susceptibility testing and the use of drugs shown to be in the susceptible category is very imprecise. This imprecision is due to the effect of host responses, site of infection, toxin production by bacteria that is independent of antimicrobial susceptibility, the presence of biofilm, drug pharmacodynamics and other factors.

Resistant: The "resistant" category implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules, and/or that demonstrate zone diameters that fall in the range where specific microbial resistance mechanisms (e.g. beta-lactamases) are likely, and clinical efficacy of the agent against the isolate has not been reliably shown in treatment studies. (CLSI definition).
Note that this definition says nothing about the chances of clinical success; in fact predicting clinical outcome based on susceptibility testing and the use of drugs shown to be in the resistant category is imprecise. This imprecision is due to the effect of host responses, site of infection, toxin production by bacteria that is independent of antimicrobial susceptibility, the presence or absence of biofilm, drug pharmacodynamics and other factors. However, with the exception of urinary bladder infections and some mycobacterial infections, most clinicians avoid the use of a "resistant" category drug to treat infection.

Intermediate: The "intermediate" category includes isolates with antimicrobial MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (e.g. quinolones and beta-lactams in urine) or when a higher than normal dosage of a drug can be used (e.g. betalactams). This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.(CLSI definition)
When susceptibility testing is done by microbroth dilution, serial dilutions of each antibiotic are inoculated with a standardized suspension of the bacteria being tested, and then monitored for growth. The minimum inhibitory concentration (MIC) for a particular bacteria/antibiotic combination is defined as the lowest concentration of antimicrobial agent in micrograms per milliliter that prevents the in vitro growth of bacteria.
Susceptibility reports include MIC data along with an interpretation of S, I, or R. In addition to the actual MIC number, other information that is critical in choosing an appropriate antibiotic includes half-life and achievable concentration at the site of infection. The physician should keep in mind that the antibiotic with the lowest MIC is not always the most appropriate choice of therapy.

Antimicrobial Susceptibility Testing Agents: Mechanism of Action
Following antibiotics, sorted by class and their mechanism of actions. The highest division is between bactericidal antibiotics and bacteriostatic antibiotics. Bactericidal kills bacteria directly where as bacteriostatic prevents them from dividing. However, these classifications are based on laboratory behavior; in practice, both of these are capable of ending a bacterial infection.

1. Class: Aminoglycosides
(Infections caused by Gram-negative bacteria, such as *Escherichia coli* and *Klebsiella* particularly *Pseudomonas aeruginosa*. Effective against Aerobic bacteria (not obligate/facultative anaerobes) and tularemia.)
Antibiotics: Amikacin, Gentamicin, Kanamycin, Neomycin, Netilmicin, Tobramycin,
Mechanism of Action: Binding to the bacterial 30S ribosomal subunit (some work by binding to the 50S subunit), inhibiting the translocation of the peptidyl-tRNA from the A-site to the P-site and also causing misreading of mRNA, leaving the bacterium unable to synthesize proteins vital to its growth.

2. Class: Carbapenems
(Bactericidal for both Gram-positive and Gram-negative organisms and therefore useful for empiric broad-spectrum

*Pharmacokinetic: Explained in Encyclopedia

antibacterial coverage. (Note MRSA resistance to this class.)
Antibiotics: Ertapenem, Doripenem, Imipenem / Cilastatin & Meropenem.
Mechanism of Action: Inhibition of cell wall synthesis.

3. Class: **Cephalosporins**
Antibiotics: First generation – Cefadroxil, Cafazolin, Cefalothin, Cefalexin (Good coverage against Gram positive infections).
Second generation - Cefaclor, Cefamandole, Cefoxitin, Cefprozil, Cefuroxime (Less gram positive cover, improved gram negative cover.)
Third generation - Cefixime, Cefdinir, Cefditoren, Cefoperazone, Cefotaxime, Cefpodoxime, Ceftazidime, Ceftibuten, Ceftizoxime (Improved coverage of Gram negative organisms, except *Pseudomonas*. Reduced Gram positive cover).
Fourth generation – Ceftriaxone (Covers pseudomonal infections)
Fifth generation- Cefepime (Used to treat MRSA).
Mechanism of Action: Disrupt the synthesis of the peptidoglycan layer of bacterial cell walls.

4. Class: **Monobactams**
Antibiotic: Aztreonam
Mechanism of Action: Disrupt the synthesis of the peptidoglycan layer of bacterial cell walls.

5. Class: **Penicillins**
(Wide range of infections; penicillin used for streptococcal infections, syphilis, and Lyme disea)
Antibiotic: Amoxicillin, Ampicillin, Azlocillin, Carbenicillin, Cloxacillin, Dicloxacillin, Flucloxacillin, Mezlocillin, Methicillin, Nafcillin, Oxacillin, Penicillin G, Piperacillin & Ticarcillin.
Mechanism of Action: Disrupt the synthesis of the peptidoglycan layer of bacterial cell walls.

6. Class: Penicillin combinations
Antibiotic: Amoxicillin/clavulanate, Ampicillin/sulbactam, Piperacillin/tazobactam, Ticarcillin/clavulanate.
Mechanism of Action: The second component prevents bacterial resistance to the first component.

7. **Class**: **Glycopeptides**
Antibiotic: Teicoplanin, Vancomycin, Telavancin
Mechanism of Action: Inhibiting peptidoglycan synthesis.

8. Class: Lincosamides (Serious staph-, pneumo-, and streptococcal infections in penicillin-allergic patients, also anaerobic infections; clindamycin topically for acne).
Antibiotic: Clindamycin, Lincomycin.
Mechanism of Action: Bind to 50S subunit of bacterial RNA thereby inhibiting protein synthesis.

9. Class: **Macrolides**
Antibiotic: Azithromycin, Clarithromycin, Dirithromycin, Erythromycin, Roxithromycin, Troleandomycin (Streptococcal infections, syphilis, upper respiratory tract infections, lower respiratory tract infections, mycoplasmal infections, Lyme disease), Telithromycin (Pneumonia) & Spectinomycin (Gonorrhea).
Mechanism of Action: Inhibition of bacterial protein biosynthesis by binding reversibly to the subunit 50S of the bacterial ribosome, thereby inhibiting translocation of peptidyl tRNA.

10. Class: Polypeptides
Antibiotic: Bacitracin, Colistin & Polymyxin B (Eye, ear or bladder infections; usually applied directly to the eye or inhaled into the lungs; rarely given by injection).
Mechanism of Action: Inhibits isoprenyl pyrophosphate, a molecule that carries the building blocks of the peptidoglycan bacterial cell wall outside of the inner membrane. Interact with the gram negative bacterial outer membrane and cytoplasmic membrane. It displaces bacterial counter ions, which destabilizes the outer membrane. They act like a detergent against the cytoplasmic membrane, which alters its permeability. Polymyxin B and E are bactericidal even in an isosmotic solution.

11. Class: Quinolones
Antibiotic: Ciprofloxacin, Enoxacin, Gatifloxacin, Levofloxacin, Lomefloxacin, Moxifloxacin, Nalidixic acid, Norfloxacin, Ofloxacin (Urinary tract infections, bacterial prostatitis, community-acquired pneumonia, bacterial diarrhea, mycoplasmal infections, gonorrhea), Trovafloxacin, Grepafloxacin, Sparfloxacin & Temafloxacin.
Mechanism of Action: Inhibit the bacterial DNA gyrase or the topoisomerase IV enzyme, thereby inhibiting DNA replication and transcription.

12. Class: **Sulfonamides**
Antibiotic: Sulfonamidochrysoidine, Sulfacetamide, Sulfadiazine, Sulfamethizole, Sulfamethoxazole, Sulfisoxazole, Trimethoprim & Trimethoprim-Sulfamethoxazole (Co-trimoxazole). Urinary tract infections (except sulfacetamide, used for eye infections, and mafenide and silver sulfadiazine, used topically for burns)
Mechanism of Action: Folate synthesis inhibition. They are competitive inhibitors of the enzyme dihydropteroate synthetase, DHPS. DHPS catalyses the conversion of PABA (para-aminobenzoate) to dihydropteroate, a key step in folate synthesis. Folate is necessary for the cell to synthesize nucleic acids (nucleic acids are essential building blocks of DNA and RNA), and in its absence cells will be unable to divide.

13. Class: Tetracyclines
Antibiotic: Doxycycline, Minocycline, Oxytetracycline & Tetracycline (Syphilis, chlamydial infections, Lyme disease, mycoplasmal infections, acne rickettsial infections, *malaria *Note: Malaria is caused by a protist and not a bacterium.).
Mechanism of Action: Inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex. They do so mainly by binding to the 30S ribosomal subunit in the mRNA translation complex.

14. Class: Drugs against mycobacteria
Antibiotics: Isoniazid (Antituberculosis), Rifampicin (Mycobacterium avium complex), Streptomycin (Antituberculosis)
Mechanism of Action: Binds to the β subunit of RNA polymerase to inhibit transcription.
The most useful means for assessing the adequacy of antimicrobial treatment in many infections is the clinical response of the patient to treatment and, if needed, the demonstration by repeated culture that the infecting organism either has been eliminated or still persists. Antibiotic susceptibility tests are intended to be a guide for the clinician, not a guarantee that an antimicrobial agent will be effective in treatment, as many other in vivo factors may alter a patient's response to therapy. Consultation with an infectious disease physician is recommended for assistance with complex antimicrobial therapy.

Methods of Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include:

Diffusion	Dilution	Diffusion&Dilution
Stokes method	Minimum Inhibitory Concentration	E-Test method
Kirby-Bauer method	(a) Broth Dilution (b)Agar Dilution	

Disc Diffusion Methods

The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-Bauer method being recommended by the National Committee for Clinical Laboratory Standards (NCCLS). The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures as described here.

Procedure

1-Inoculum Preparation

1.1 Growth Method

1. At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth.
2. The broth culture is incubated at 35^{0}C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours).
3. The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 10^{8}CFU/ml for *E.coli* ATCC 25922. To perform this step properly, either a photometric device can be used or, if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

1.2-Direct Colony Suspension Method

1. As a convenient alternative to the growth method, the inoculum can be prepared by making a direct broth or saline suspension of isolated colonies selected from a 18- to 24-hour agar plate (a nonselective medium, such as blood agar, should be used). The suspension is adjusted to match the 0.5 McFarland turbidity standard, using saline and a vortex mixer.
2. This approach is the recommended method for testing the fastidious organisms, *Haemophilus*spp., *N. gonorrhoeae*, and streptococci, and for testing staphylococci for potential methicillin or oxacillin resistance.

2-Inoculation of Test Plates

1. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.
2. The dried surface of a Müeller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60^0 each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.
3. The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.
   NOTE: Extremes in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

3-Application of Discs to Inoculated Agar Plates

1. The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface.
2. The plates are inverted and placed in an incubator set to 35^{0}C within 15 minutes after the discs are applied. With the exception of *Haemophilus* spp., streptococci and *N. gonorrhoeae*, the plates should not be incubated in an increased CO_2 atmosphere, because the interpretive standards were developed by using ambient air incubation, and CO_2will significantly alter the size of the inhibitory zones of some agents.

4-Reading Plates and Interpreting Results

1. After 16 to 18 hours of incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum was too light and the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petri plate. The petri plate is held a few inches above a black, nonreflecting background and illuminated with reflected light. If blood was added to the agar base (as with streptococci), the zones are measured from the upper surface of the agar illuminated with reflected light, with the cover removed. If the test organism is a *Staphylococcus* or *Enterococcus* spp., 24 hours of incubation are required for vancomycin and oxacillin, but other agents can be read at 16 to 18 hours. Transmitted light (plate held up to light) is used to examine the oxacillin and vancomycin zones for light growth of methicillin- or vancomycin- resistant colonies, respectively, within apparent zones of inhibition. Any discernable growth within zone of inhibition is indicative of methicillin or vancomycin resistance.
2. The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. However, discrete colonies growing within a clear zone of inhibition should be subcultured, re-identified, and retested. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., the thin veil of swarming growth in an otherwise obvious zone of inhibition should be ignored. When using blood-supplemented medium for testing streptococci, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth), and measure the more obvious margin to determine the zone diameter.
   The sizes of the zones of inhibition are interpreted by referring to Tables 2A through 2L (Zone Diameter Interpretative

Standards and equivalent Minimum Inhibitory Concentration Breakpoints) of the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement, and the organisms are reported as either susceptible, intermediate, or resistant to the agents that have been tested. Some agents may only be reported as susceptible, since only susceptible breakpoints are given.

Dilution Methods

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism. This can be achieved by dilution of antimicrobial in either agar or broth media. Antimicrobials are tested in $\log_2$serial dilutions (two fold).

Minimum Inhibitory Concentration (MIC)

Diffusion tests widely used to determine the susceptibility of organisms isolated from clinical specimens have their limitations; when equivocal results are obtained or in prolonged serious infection e.g. bacterial endocarditis, the quantitation of antibiotic action vis-a-vis the pathogen needs to be more precise. Also the terms 'Susceptible' and 'Resistant'can have a realistic interpretation. Thus when in doubt, the way to a precise assessment is to determine the MIC of the antibiotic to the organisms concerned.

There are two methods of testing for MIC:
(a) Broth dilution method (b) Agar dilution method.

(a) Broth Dilution Method

The Broth Dilution method is a simple procedure for testing a small number of isolates, even single isolate. It has the added advantage that the same tubes can be taken for MBC tests also:

Materials

Sterile graduated pipettes of 10ml, 5ml, 2ml and 1ml Sterile capped 7.5 x 1.3 cm tubes / small screw-capped bottles, Pasteur pipettes, overnight broth culture of test and control organisms (same as for disc diffusion tests), required antibiotic in powder form (either from the manufacturer or standard laboratory accompanied by a statement of its activity in mg/unit or per ml. Clinical preparations should not be used for reference technique.), required solvent for the antibiotic, sterile Distilled Water -500ml and suitable nutrient broth medium.
Trimethoprim and sulphonamide testing requires thymidine free media or addition of 4% lysed horse blood to the media. A suitable rack to hold 22 tubes in two rows i-e 11 tubes in each row.

Stock solution

Stock solution can be prepared using the formula
(1000/P) x V x C= W
Where P=Potency given by the manufacturer in relation to the base
V= Volume in ml required
C=Final concentration of solution (multiples of 1000)
W= Weight of the antimicrobial to be dissolved in the volume V
Note:the stock solutions are made in higher concentrations to maintain their keeping qualities and stored in suitable aliquots at -20°.Once taken out, they should not be refrozen or reused.

Method

Prepare stock dilutions of the antibiotic of concentrations 1000 and 100 µg/L as required from original stock solution (10,000mg/L). Arrange two rows of 12 sterile 7.5 x1.3 cm capped tubes in the rack. In a sterile 30ml (universal) screw capped bottle, prepare 8ml of broth containing the concentration of antibiotic required for the first tube in each row from the appropriate stock solution already made. Mix the contents of the universal bottle using a pipette and transfer 2ml to the first tube in each row. Using a fresh pipette add 4 ml of broth to the remaining 4 ml in the universal bottle mix and transfer 2ml to the second tube in each row. Continue preparing dilutions in this way but where as many as 10 or more are required the series should be started again half the way down. Place 2ml of antibiotic free broth to the last tube in each row. Inoculate one row with one drop of an overnight broth culture of the test organism diluted approximately to 1 in 1000 in a suitable broth and the second row with the control organism of known sensitivity similarly diluted. The result of the test is significantly affected by the size of the inoculum. The test mixture should contain 10^6/ml. If the broth culture used has grown poorly, it may be necessary to use this undiluted. Incubate tubes for 18 hours at 37°C. Inoculate a tube containing 2ml broth with the organism and keep at 4°C in a refrigerator overnight to be used as standard for the determination of complete inhibition.

Reading of result

MIC is expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube. Because very faint turbidity may be given by the inoculum itself, the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition. Standard strain of known MIC value run with the test is used as the control to check the reagents and conditions.

The Agar dilution Method

Agar dilutions are most often prepared in petri dishes and have advantage that it is possible to test several organisms on each plate .If only one organism is to be tested e.g *M.tuberculosis*, the dilutions can be prepared in agar slopes but it will then be necessary to prepare a second identical set to be inoculated with the control organism. The dilutions are made in a small volume of water and added to agar which has been melted and cooled to not more than 60°C. Blood may be added and if 'chocolate agar' is required, the medium must be heated before the antibiotic is added.
It would be convenient to use 90 mm diameter petri dishes and add one ml of desired drug dilutions to 19 ml of broth. The factor of agar dilution must be allowed for in the first calculation as follows.

Final volume of medium in plate	=	20 ml
Top antibiotic concentrations	=	64mg/l
Total amount of drug	=	1280µg to be added to 1 ml of water
2ml of 1280 µg /ml will be required to start the dilution	=	2560µg in 2 ml
	=	1.28ml of 2000µg /ml ±0.72 ml of water.

1 ml of this will be added to 19 ml agar.
(Note stock dilution of 2000µg /ml is required for this range of MIC)

Reading of result

The antibiotic concentration of the first plate showing ≥ 99% inhibition is taken as the MIC for the organism.

Dilution and Diffusion

E test also known as the epsilometer test is an 'exponential gradient' testing methodology where 'E' in E test refers to the

Greek symbol epsilon (ε). The E test(AB Biodisk) which is a quantitative method for antimicrobial susceptibility testing applies both the dilution of antibiotic and diffusion of antibiotic into the medium. A predefined stable antimicrobial gradient is present on a thin inert carrier strip. When this E test strip is applied onto an inoculated agar plate, there is an immediate release of the drug. Following incubation , a symmetrical inhibition ellipse is produced. The intersection of the inhibitory zone edge and the calibrated carrier strip indicates the MIC value over a wide concentration range (>10 dilutions) with inherent precision and accuracy.

E test can be used to determine MIC for fastidious organisms like *S. pneumoniae*, β-hemolytic streptococci, *N. gonorrhoeae*, *Haemophilus* sp. and anaerobes. It can also be used for Nonfermenting Gram Negative bacilli (NFGNB) for eg- *Pseudomonas* sp. and *Burkholderia pseudomallei*. Resistance of major consequence may be detected for e.g., the test is very useful in detecting glycopeptide resistant Enterococci (GRE) and glycopeptide intermediate *S. aureus* (GISA) and slow growing pathogens such as *Mycobacterium tuberculosis.* Further it can be used for detection of extended spectrum beta lactamases (ESBL). In conclusion E test is a simple, accurate and reliable method to determine the MIC for a wide spectrum of infectious agents.

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Encyclopedia

Pharmacokinetics provides a mathematical basis to assess the time course of drugs and their effects in the body. Pharmacokinetics is the study of drug absorption, distribution within body, and drug elimination: Absorption depends on the route of administration. Drug distribution depends on how soluble the drug molecule is in fat (to pass through membranes) and on the extent to which the drug binds to blood proteins (albumin). Drug elimination is accomplished by excretion into urine and/or by inactivation by enzymes in the liver.

These pharmacokinetic processes, often referred to as ADME (Absorption, Distribution, Metabolism, and Excretion), determine the drug concentration in the body when medicines are prescribed. A fundamental understanding of these parameters is required to design an appropriate drug regimen for a patient. The effectiveness of a dosage regimen is determined by the concentration of the drug in the body.

Ideally, the concentration of drug should be measured at the site of action of the drug; that is, at the receptor. However, owing to inaccessibility, drug concentrations are normally measured in whole blood from which serum or plasma is generated. Other body fluids such as saliva, urine and cerebrospinal fluid (CSF) are sometimes used. It is assumed that drug concentrations in these fluids are in equilibrium with the drug concentration at the receptor.

In routine clinical practice, serum drug level monitoring and optimization of a dosage regimen require the application of clinical pharmacokinetics. A number of drugs show a narrow therapeutic range and for these drugs therapeutic drug level monitoring is required.

Routes of Administration

Parenterally: Intravenous, Intramuscular, Subcutaneous.

Others: Orally, Rectally, Inhalation: Absorption through mucous membranes, Topical.

Analysis: Pharmacokinetic (PK) analysis is performed by noncompartmental (model independent) or compartmental methods. Noncompartmental methods estimate the exposure to a drug by estimating the area under the curve of a concentration-time graph. Compartmental methods estimate the concentration-time graph using kinetic models. Compartment-free methods are often more versatile in that they do not assume any specific compartmental model and produce accurate results also acceptable for bioequivalence studies.

Noncompartmental analysis: Noncompartmental PK analysis is highly dependent on estimation of total drug exposure. Total drug exposure is most often estimated by area under the curve (AUC) methods, with the trapezoidal rule (numerical integration) the most common method. Due to the dependence on the length of 'x' in the trapezoidal rule, the area estimation is highly dependent on the blood/plasma sampling schedule. That is, the closer time points are, the closer the trapezoids reflect the actual shape of the concentration-time curve.

Compartmental analysis: Compartmental PK analysis uses kinetic models to describe and predict the concentration-time curve. PK compartmental models are often similar to kinetic models used in other scientific disciplines such as chemical kinetics and thermodynamics. The advantage of compartmental over some noncompartmental analyses is the ability to predict the concentration at any time. The disadvantage is the difficulty in developing and validating the proper model. Compartment-free modeling based on curve stripping does not suffer this limitation. The simplest PK compartmental model is the one-compartmental PK model with IV bolus administration and first-order elimination. The most complex PK models (called PBPK models) rely on the use of physiological information to ease development and validation.

Bioanalytical methods: Bioanalytical methods are necessary to construct a concentration-time profile. Chemical techniques are employed to measure the concentration of drugs in biological matrix, most often plasma. Proper bioanalytical methods should be selective and sensitive.

Importance of Pre-cleaning and Disinfection of Surgical Instruments

Introduction

To understand why it is important to Pre-clean and Disinfect the Surgical Instruments, it is necessary to know the past incidents that occurred when this criterion was neglected and the problems encountered. Also, learn about their sources and the different ways by which these microorganisms have survived the disinfection procedure and caused infection. Though the problem has been recognized for decades, the number of reports of pathogenic mycobacterial disease caused by the use of contaminated devices or from an invasive procedure has been increasing.(Carson *et al.* ; Schulze-Röbbecke *et al.*; Fujita *et al.*). There are several excellent reviews on nosocomial outbreaks/pseudo-outbreaks that have been published by (Fraser 1981; Wallace *et al.*; Phillips & von Reyn 2001).

Potential situations associated with **Pathogenic Environmental Mycobacteria (PEM)** isolation from a clinical specimen is explained in this table. (Adapted from Phillips & von Reyn 2001).

Situation	Definition
Colonization	Isolation of potentially Pathogenic EM without signs or symptoms of disease attributed to the organism.
Infection/disease	Clinical evidence of infection attributed to the organism.
Pseudo-infection	No evidence of infection or colonization and isolation in the laboratory has resulted from contamination of the environment (can occur at any point: from a contaminated device, in obtaining the specimen, or up through the final cultivation in the laboratory).

Investigation

From 1956 to 1979, CDC carried out 252 hospital outbreak investigations; these have been summarized by (Stamm and co-workers). In the ensuing 16 years through 1995, CDC assisted in another 193 outbreak investigations. In the early years (1956-1962), the two most common problems investigated were epidemics of gastrointestinal disease, primarily due to Salmonella species or enteropathogenic *E. coli*, or staphylococcal infections. Increasing numbers of HAI outbreaks were associated with the bloodstream, respiratory tract, urinary tract, and surgical wounds or medical devices. These included outbreaks of hepatitis A virus or hepatitis B virus infections; necrotizing enterocolitis in nurseries; sternal wound infections after open heart surgery, particularly those caused by rapidly growing mycobacteria; and nosocomial Legionnaires' disease. Also during this period, CDC recorded increasing numbers of outbreaks associated with microorganisms resistant to multiple antimicrobials, particularly aminoglycoside-resistant *Enterobacteriaceae* and non-fermentative gram-negative bacilli, and MRSA.

Outbreaks of Infection

Fifty-two (46%) of the 114 outbreaks were associated with either an invasive device or invasive procedure. Dialyzers (10; 43%) were the most common invasive devices associated with outbreaks followed by needleless intravascular device use among patients in inpatient, outpatient, or home care settings (7; 29%). The most common invasive procedures were surgery (21; 50%), dialysis (16; 37%), or cardiac catheterization (3; 7%). Twenty (17.5%) of the 114 outbreak investigations were associated with contaminated products, including intravenous anesthetics (9; 8.0%), parenteral solutions (5; 4.4%), or blood products (2; 1.8%). Twenty-one (28.6%) of the infectious disease outbreaks were associated with multidrug-resistant organisms, including multidrug-resistant *M. tuberculosis*; VRE; *S. aureus* with reduced susceptibility to vancomycin, vancomycin-resistant *Staphylococcus* epidermidis, or extended spectrum beta-lactamase producing E. coli and K. pneumoniae.

In hospital-wide data, UTIs have accounted for approximately 40% of all HAIs, but UTIs make up a smaller proportion of HAIs occurring in the ICU setting. UTIs account for 15-21% of HAIs in pediatric ICU patients, 23% of HAIs in adult U.S. ICU patients, and 18% of ICU infections in the European EPIC study. The prevalence of UTI varies by ICU type; rates of CA-UTIs reported through the Centers for Disease Control and Prevention's (CDC) National Nosocomial Infections Surveillance (NNIS) system between January 2002 and June 2004 ranged from 3.0 infections/1,000 catheter-days in cardiothoracic ICUs to 6.7 infections/1,000 catheter-days in burn or neurosurgical ICUs. The rate of UTI in pediatric ICUs was 4.0 infections/1,000 catheter-days, lower than the rate seen in an equivalent adult medical ICU population of 5.1 infections/1,000 catheter-days. Nosocomial UTI is infrequently identified in neonatal ICUs. In data collected in a non-ICU setting in 42 German hospitals, the rate of infection was similar, 6.8 infections per 1,000 urinary-catheter days.

From October 1986 through June 1988, at a hospital in Wisconsin, USA, *Pseudomonas aeruginosa* of the biliary and respiratory tract, or bloodstream occurred in 16 (6.7%) of 240 patients undergoing Endoscopic Retrograde Cholangio-Pancreatography (ERCP) and in 99 (8.9%) of 1109 patients undergoing other upper gastrointestinal (UGI) endoscopic procedures. The endoscopes were routinely reprocessed in automated reprocessing machine that flushed with a detergent solution, disinfected with one of two liquid chemical germicides (2% glutaraldehyde; 2% glutaraldehyde/ 7.05% phenol/ 1.2% sodium phenate diluted 1:16 in tap water). An investigation performed by the hospital in June 1988 indicated that a thick biofilm of *P. aeruginosa* had formed in the detergent holding tank, inlet water hose, and air vents of the automated machine. Attempts to disinfect the machine by the manufacturer's instructions using commercial preparations of glutaraldehyde were unsuccessful.

Sources of Infection

● *Infection of intravascular catheters, pacemakers*

Vascular devices such as indwelling venous access lines and vascular shunts can become infected with both **Rapidly Growing Mycobacteria (RGM)** and other species (Katz *et al.*; Schinsky *et al.*; Rodriguez-Gancedo *et al.*; Bouza *et al.*). Infections can result in skin and soft tissue involvement (including tunnel site infections) with or without bacteraemia. Pocket infections involving pacemakers have also been seen with PEM, with **MAC (both *M. avium M. intracellulare*)** and especially *M. abscessus* (Amin *et al.*; Katona *et al.*; Cutay *et al.*; Verghese *et al.*).

● *Dialysis related infection*

Dialysis related PEM infection has been reported in both intravascular and peritoneal mechanisms of renal replacement therapy. Rapidly growing mycobacteria species are the most

commonly implicated. Contaminated aqueous solutions used to sterilize the re-usable dialysis filters have been involved in many cases. PEM representatives have been isolated from water supplies of haemodialysis centres (Carson *et al.*). In 1982, 27/140 patients receiving haemodialysis developed infection after being exposed to mycobacteria in water used to prepare dialysis fluids (Bolan *et al.*). Peritonitis can occur in patients undergoing chronic ambulatory peritoneal dialysis (Band *et al.*). In this setting it can involve the catheter insertion site, tunneling tract and/or the peritoneum itself. **MAC (both *M. avium M. intracellulare*)** and the rapid growers are the most commonly isolated species (Band *et al.*; Soriano *et al.*; Lowry *et al*. 1990; Vera & Lew 1999). Patients with end stage renal disease from any cause are likely to be more prone to infection due to impaired lymphocyte and neutrophil activity in the face of uraemia. Catheter removal improves the rate of cure and antibiotics are necessary to prevent clinical failure. Sequelae include adhesions and sometimes difficulty replacing the catheter (Hakim *et al.*).

● *Bronchoscopy and Endoscopy*

An estimated 497,000 bronchoscopy procedures were performed in the United States in 1996. Several outbreaks have highlighted the problems of pulmonary infection and false-positive culture results because of inadequately cleaned fiberoptic bronchoscopes. So the need is realized for higher-level disinfection and sterilization of these scopes, especially after use on patients who could have tuberculosis. Although the hospital's procedures for disinfection, corresponded with most guidelines, the bronchoscope showed patient debris after disinfection, indicating that the manual cleaning was inadequate and was not approved for reprocessing in the hospital's automated endoscope reprocessor system. Failure to perform leak testing led to failure to discover a hole in the sheath of a bronchoscope, which led to inadequate disinfection and transmission of *M. tuberculosis* to patients via the bronchoscope resulting in infection and pseudoinfections. One outbreak was believed to be a result of a **manufacturing defect** of the biopsy-port caps, and another was due to **incorrect connectors** joining the bronchoscope suction channel to the Steris System processor, obstructing peracetic acid flow through the bronchoscope lumen. Infection and pseudoinfection from bronchoscopic procedures reflect a number of problems including **ineffective cleaning** due to poor technique, damaged equipment, **difficult-to-clean accessories, ineffective reprocessing,** use of tap water to rinse the scopes, **inappropriate storage** (e.g., coiling the scopes), and **lack of familiarity** with national recommendations for reprocessing. (Srinivasan et al.) distributed a survey to practicing bronchoscopists regarding infection control issues related to bronchoscopy and specific reprocessing recommendations.

Medical directors of bronchoscopy suites or attending bronchoscopists completed 46 surveys. “Of the respondents, 65% were not familiar with national reprocessing recommendations, and 39% did not know what reprocessing procedure was used at their own institution.” In addition, some parts of the bronchoscopes (e.g., reusable spring-operated suction valves) could require autoclaving if they become heavily contaminated with microbes that are relatively resistant to disinfection such as mycobacteria. Working and suction channels of 241 flexible gastrointestinal endoscopes at 80 healthcare facilities, it was found that 47% (38/80) of facilities had at least one patient-ready endoscope whose suction or biopsy channels were visibly encrusted with debris, and 11% (26/241) of endoscopes had severely scratched channels that provided pockets for debris. Only 5.4% (3/56) of facilities that attempted to dry their endoscopes between procedures were successful. Because high-level disinfectants require clean surfaces, flexible endoscopes must be carefully cleaned of all mucus, blood, and other biologic materials before subjecting them to a high-level disinfectant. To further complicate endoscope care, automated machines developed for endoscope reprocessing have been flawed. Users should adhere carefully to the manufacturer's protocols but also should be aware of the possibility that colonization of the washer holding tanks is not reversible despite use of the manufacturer's recommended disinfection protocol. Surveillance for endoscope-related infection and pseudoinfection is important, and infection control practitioners must educate their endoscope users (e.g., endoscopy suite personnel and physicians) about problems discussed in this section; the users also must be vigilant to monitor best practice for a complicated cleaning procedure because there are many opportunities for inadequate disinfection.

● *Other medical and surgical procedures*

Post-surgical infections with PEM have been reported in many settings (Robicsek *et al.*, 1978, 1988; Hoffman *et al*. 1981; Safranek *et al.*; Wallace *et al.*; Jarvis 1991; Grange 1992; Syed *et al.*). They typically arise from solutions or instruments that have been inadequately sterilized (Phillips & von Reyn 2001). Mediastinitis and sternal wound infections due to *M. fortuitum M. chelonae* been reported after cardiothoracic surgery (Hoffman *et al.*; Kuritsky *et al.*). Infection due to *M. chelonae* felt to have resulted by transmission between patients from contaminated instruments (Lowry *et al.*). Spinal infections have been reported after epidural injections and also after spinal surgery. A large outbreak after discovertebral surgery in France involving 58 patients in a single hospital in Paris occurred over a period of more than 10 years. This was a result of chronic contamination of the hospital water supply with *M. xenopi* (Astagneau *et al.*). Other surgical procedures reported to result in PEM infection include augmentation mammoplasty, liposuction, laser vision correction surgery and other plastic surgical procedures (Murillo *et al.*). *M. abscessus, M. chelonae M. fortuitum* major. In addition to solutions, instrumentation and implants that have been implicated include lacrimal duct probes, tympanostomy tubes, epidural catheters and graft materials.

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John Franklin Enders

Born: 10 February 1897, West Hartford, CT, USA
Died: 8 September 1985, Waterford, CT, USA

Affiliation at the time of the award: Harvard Medical School, Boston, MA, USA, Research Division of Infectious Diseases, Children's Medical Center, Boston, MA, USA Prize motivation: "for their discovery of the ability of poliomyelitis viruses to grow in cultures of various types of tissue"

John Franklin Enders was born on February 10th, 1897, at West Hartford, Connecticut, U.S.A. He is the son of John Ostrom Enders, a banker in Hartford, and Harriet Goulden Enders (*née* Whitmore).

Enders was educated at the Noah Webster School at Hartford and St. Paul's School in Concord, New Hampshire. Finishing school in 1915, he went to Yale University, but in 1917 left his studies there to become, in 1918, a pilot in the U.S. Air Force with the rank of Ensign. After the First World War he returned to Yale and was given, in 1919, the degree of B.A. (*honoris causa*) and the normal degree in 1920.

He then went into business in real estate in Hartford, but, becoming dissatisfied with this, he entered Harvard University. For four years he studied English literature and Germanic and Celtic languages with the idea of becoming a teacher of English, but he was not satisfied with this career either. He had been for a long time interested in biology and this interest was reawakened by his friendships with medical students at Harvard, with the result that he decided to enter as a candidate for the Ph.D. degree in bacteriology and immunology. In coming to this decision he was influenced by the late Professor Hans Zinsser, who was then Head of the Department of Bacteriology and Immunology at Harvard and by Dr. H. K. Ward, who later became Professor of Bacteriology at the University of Sidney, Australia.

In 1930, Enders received the degree of Ph.D. at Harvard for a thesis which presented evidence that bacterial anaphylaxis and hypersensitivity of the tuberculin type are distinct phenomena.

From 1930 until 1946, Enders remained at Harvard as a member of the teaching staff. During this period he studied, first, the elucidation of certain factors related to bacterial virulence and the resistance of the host organism. He then clarified, in collaboration with Ward, Shaffer, Wu, and others the inhibitory effect of the type specific capsular polysaccharides of *Pneumococcus* upon the phagocytic process. This work discovered a new form of Type I polysaccharide and produced evidence that complement played a catalytic-like part in the opsonization of bacteria by specific antibody.

In 1938, Enders began the study of some of the mammalian viruses, and undertook, in 1941, in collaboration with Cohen, Kane, Levens, Stokes and others, a study of the virus of mumps. This work provided serological tests for the diagnosis of this disease and a skin test for susceptibility to it, and demonstrated the immunizing effect of inactivated mumps virus and the possibility of attenuating the virulence of this virus by passing it through chick embryos. It was shown that mumps often occurs in a form that is not apparent, but nevertheless confers a resistance which is as effective as that conferred by the visible disease.

In 1946, Enders was asked to establish a laboratory for research in infectious diseases at the Children's Medical Center at Boston. In this laboratory much outstanding work on the viral diseases of man has been done under his direction and it was here that the work was done on the cultivation of the poliomyelitis viruses for which Enders was awarded, together with T. H. Weller and F. C. Robbins, the Nobel Prize for Physiology or Medicine in 1954.

Since this time Enders has returned, in collaboration with Peebles, to his earlier work on measles. He is now Higgins University Professor at Harvard University and Chief of the Research Division of Infectious Diseases of the Children's Hospital, Boston, Massachusetts, U.S.A.

Enders is a member of a great number of American learned societies, the Society for General Microbiology and the Royal Society for the Promotion of Health in Great Britain, the Deutsche Akademie der Naturforscher (Leopoldina), and is Foreign Corresponding Member of the British Medical Association and the Académie Royale de Médicine de Belgique.

He married Sarah Frances Bennett, of Brookline, Massachusetts, in 1927. She died in 1943, and in 1951 Enders married Carolyn B. Keane of Newton Center, Massachusetts. He has one son John Ostrom Enders II, one daughter, Sarah Enders, and a stepson, William Edmund Keane.

MAJOR AWARDS AND DISTINCTIONS

1953 Election to National Academy of Sciences
1953 Passano Award
1954 Lasker Award
1954 Nobel Prize in Physiology or Medicine
1955 Charles V. Chapin Medal
1955 Gordon Wilson Medal
1961 TIME Man of the Year
1962 Robert Koch Medaille, Germany
1963 Presidential Medal of Freedom, United States
1967 Foreign Member, Royal Society of London
1981 Galen Medal of the Worshipful Society of Apothecaries, London.
Honorary doctoral degrees from thirteen universities.

John F. Enders died on September 8, 1985.

Reference:

http://nobelprize.org/nobel_prizes/medicine/laureates/1954/enders.html

Enjoy the humour

Hilarious Medical Terminology

BIRDWATCHERS TWITCH: The nervous excitement of spotting a species for the first time. {New Scientist, 1982}

CHRISTMAS DEPRESSION: Psychological stress during holidays related to the use of alcohol and social pressures. {JAMA, 1982}

CREDIT-CARD-ITIS: Pain over the rear and down thigh due to pressure on nerve from a wallet stuffed with credit cards. {New England Medical Journal, 1966}

HOUSWIFITIS: Nervous symptoms related to spending too much time managing a busy household. {Centrescope, 1976}

REFLEX HORN SYNDROME: Tendency for drivers waiting in traffic jams to toot horns. {New England Medical Journal, 1976}

RETIRED HUSBAND SYNDROME: Tension, headaches, depression and anxiety felt by women whose husbands have just retired. {Western Journal of Medicine, 1984}

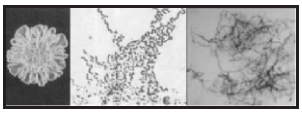
The following is a little known, true story about Albert Einstein.

Albert Einstein was just about finished his work on the theory of special relativity, when he decided to take a break and go on vacation to Mexico. So he hopped on a plane and headed to Acapulco. Each day, late in the afternoon, sporting dark sunglasses, he walked in the white Mexican sand and breathed in the fresh Pacific sea air. On the last day, he paused during his stroll to sit down on a bench and watch the Sun set. When the large orange ball was just disappearing, a last beam of light seemed to radiate toward him. The event brought him back to thinking about his physics work. "What symbol should I use for the speed of light?" he asked himself. The problem was that nearly every Greek letter had been taken for some other purpose. Just then, a beautiful Mexican woman passed by. Albert Einstein just had to say something to her. Almost out of desperation, he asked as he lowered his dark sunglasses, "Do you not zink zat zee speed of light is zery fast?" The woman smiled at Einstein (which, by the way, made his heart sink) and replied, "Si" which means yes.

And now we know the symbol for the speed of light is "c".

Hilarious Quotes

- I can resist everything except temptation.
  Oscar Wilde
- Get the facts first. You can distort them later.
  Mark Twain
- Any fool can criticize, condemn and complain and most fools do.
  Benjamin Franklin
- Everyone has photographic memory; some just don't have the film.
- Always forgive your enemies - Nothing annoys them so much.
- The road to success is always under construction.
- The only reason people get lost in thought is because it's unfamiliar territory.
- There are three sides to any argument: your side, my side and the right side.
- Never argue with a fool. People might not know the difference.
- When you're right, no one remembers. When you're wrong, no one forgets.
- If you can't see the bright side of life, polish the dull side.
- Confucius's once said, "When you breathe, you inspire, and when you do not breathe, you expire."



Nocardia species

Historically, *N. asteroides* was defined biochemically as including those isolates of Nocardia that do not decompose xanthine, tyrosine, and casein. In 1888, on Guadeloupe Island, Edmond Nocard isolated anaerobic filamentous organism from lesions in cattle suffering from farcy. In 1889, Trevisan created the genus Nocardia to accommodate Nocard's isolate and named it *Nocardia farcinica*. One year later, in 1890, Eppinger isolated a similar aerobic, branching filamentous organism from a human with a fatal brain abscess and called this organism Cladothrix asteroides; however, in 1896 it was renamed *Nocardia asteroides*. Because of confusion regarding the identity of Nocard's original isolate combined with evidence that Mycobacterium causes bovine farcy, the identification of *N.farcinica* was placed in doubt. As a result, *N.farcinica* was officially removed as the type species and *N. asteroides* was designated as the new type species for the genus Nocardia.

Nocardia bacteria are weakly-Gram positive, catalase positive aerobic bacteria belonging to the sub order, Corynebacterineae, of the Actinobacteria. Nocardia are saprophytes and are present in soil water, or decaying organic. It forms partially acid-fast beaded branching filaments (acting as fungi, but being truly bacteria). The genus contains at least 25 species, of which 13 are pathogenic (Nocardiosis) to humans to both immune competent and immune compromised individuals. Nocardiosis is an acute, subacute, or chronic infectious disease that generally occurs in cutaneous, pulmonary, and disseminated forms. Primary cutaneous condition manifests as cellulitis, abscess, or lympho-cutaneous nocardiosis conditions. Pleuropulmonary nocardiosis can be acute, subacute or chronic pneumonitis in immune compromised patients as well as individuals on long term corticosteroid therapy for lung disease. Subcutaneous and lymphocutaneous infections arise from traumatic inoculation. The lymphocutaneous infection is accompanied with regional lymphoadenopathy and may drain purulent material.

Culture and Staining

Nocardia colonies have a variable appearance, but most species appear to have aerial hyphae when viewed with a dissecting microscope, particularly when they have been grown on nutritionally-limiting media. *Nocardia* grow slowly on non-selective culture media, and are strict aerobes with the ability to grow in a wide temperature range. Some species are partially acid fast (meaning that a less concentrated solution of sulfuric or hydrochloric acid should be used during the staining procedure) due to the presence of intermediate-length mycolic acids in their cell wall. Majority of strains possess the cord factor (trehalose 6-6' dimycolate) an important virulence factor. They are catalase positive and can grow easily on the most commonly used media with colonies becoming evident in 3–5 days. However sometimes prolonged incubation periods (2–3 weeks) are needed. The genus includes at least 30 different species with ten of them isolated from humans.

Virulence

The various species of *Nocardia* pathogenic bacteria with low virulence; therefore clinically significant disease most frequently occurs as an opportunistic infection in those with a weak immune system, such as small children, the elderly, and the immuno-compromised (most typically, HIV). Nocardial virulence factors are the enzymes catalase and superoxide dismutase (which inactivate reactive oxygen species that would otherwise prove toxic to the bacteria), as well as a "cord factor" (which interferes with phagocytosis by macrophages by preventing the fusion of the phagosome with the lysosome).

Cause

Nocardia infection (Nocardiosis) is a bacterial infection that usually starts in the lungs. It may spread to other organ systems - most often the brain and the skin. It may also involve the kidneys, joints, heart, eyes, and bones. Nocardia disease can get by inhaling contaminated dust or if soil containing Nocardia bacteria gets into an open wound. While individuals with normal immune systems can get this infection, the main risk factors for getting nocardiosis are a weakened immune system or chronic lung disease. People on long-term steroid therapy, those with cancer, organ or bone marrow transplants, or HIV/AIDS are at higher risk.

Symptoms

Nocardia disease symptoms vary and depend on the organs involved.

Lungs (pulmonary nocardiosis): Chest pain when breathing (may occur suddenly or slowly), Coughing up blood, Fevers, Night sweats & Weight loss,

Brain (cerebral nocardiosis): Fever, Headache & Seizures

Skin: May become chronically infected (mycetoma) and develop draining tracts, Ulcers or nodules with infection sometimes spreading along lymph nodes

Some people with nocardia infection have no symptoms.

Exams and Tests

Nocardiosis is diagnosed using tests that identify the bacteria. Depending on the part of the body infected, testing may involve taking a tissue sample by: Brain biopsy, Bronchoscopy, Lung biopsy, Skin biopsy & Sputum culture.

Treatment

Since the 1940s the sulfonamides have been the treatment of choice for the treatment of nocardiosis, but not effective in all patients.

The combination of sulfamethoxazole (SMX) with trimethoprim (TMP) is often used for treatment of nocardiosis. In a recent review of nocardiosis in patients with AIDS, the majority of these patients were unresponsive to TMP-SMX.

Most clinicians recommend a three-drug regimen consisting of TMP-SMX, amikacin, and either ceftriaxone or imipenem for patients with serious disease, CNS disease, and/or disseminated disease. Sulfonamides (or sulfamethoxazole-trimethoprim combination) remain the drug of choice for all forms of Nocardia, and the use of amikacin plus a beta-lactam ensures that all isolates (species) will be susceptible to at least one other drug (no species are resistant to both amikacin and a beta-lactam,

Of the tetracyclines, minocycline has the best in vitro activity and has been used for patients who are allergic to sulfonamides. In one study, a 400- to 600-mg daily regimen of minocycline was successfully used in the treatment of pulmonary nocardiosis in a patient who failed prior therapy with TMP-SMX.

Recently, a new class of antibiotics, the oxazolidinones, was introduced. Linezolid, the first agent in this class to be studied against Nocardia, showed extraordinary in vitro activity against all the major clinically significant species of Nocardia. The MIC90 for linezolid was 4 µg/ml. Thus this agent was successfully used to treat patients with nocardiosis, including patients who had failed therapy with other agents.

Clinical disease and microbiological diagnosis

Nocardia asteroides is the species of *Nocardia* frequently infecting humans, and most cases occur as an opportunistic infection in immunocompromised patients. Other species of medical interest are *N. brasiliensis N. caviae*. Because it is acid-fast to some degree, it stains only weakly gram positive. The most common form of human nocardial disease is a slowly progressive pneumonia, whose common symptoms include cough, dyspnea (shortness of breath), and fever. It is not uncommon for this infection to spread to the pleura or to chest wall. Pre-existing pulmonary disease, especially pulmonary alveolar proteinosis, increases the risk of contracting a *Nocardia* . Every organ can be affected if a systemic spread takes place. *Nocardia*spp are deeply involved in the process of endocarditis as one of its main pathogenic effects. In about 25-33% of people *Nocardia* will take the form of encephalitis and/or brain abscess formation.

N. asteroides and brasiliensis are causal in most cases of infection. Immune compromised patients have been reported to have the following species associated with their infection: cyriacigeorgica (endocarditis); farcinica (adrenal glands); abscessus (grain abscess) and other infections (otitidiscavarium, transvalensis). Cutaneous and systemic nocardiosis in patients with long term corticosteroid therapy has been reported for the species brasiliensis and otitidiscavarium.

Nocardiosis is an acute to chronic suppurative (formation or discharge of pus) disease caused by the aerobic gram positive filamentous bacteria, Nocardia. Primary infection is usually pulmonary, but hematogenous spread to other organs may occur. the nocardiae are ubiquitous in the environment, the isolation of these organisms may be indicative of laboratory contamination or, especially in respiratory samples, may indicate colonization and not invasive infection. Furthermore, the nocardiae may exist as saprophytes on the skin and in the upper respiratory tract. The incidence of Nocardial mycetoma in Indian reports varies from 5.2 percent to 35 percent.

Pulmonary Disease

Most Nocardia infections are believed to be acquired by inhalation of airborne spores or mycelial fragments from environmental sources. The most common manifestation of nocardial disease is pulmonary nocardiosis, occurring most frequently in immune-compromised and chronic lung disease patients. Pulmonary nocardiosis may present as a subacute or indolent disease, with symptoms present from one to several weeks. Cough is usually predominant, and patients may produce thick purulent sputum. Fever, weight loss, and malaise similar to the symptoms of nontuberculous mycobacterial disease may also be common.

Extrapulmonary Disease

One-half of all cases of pulmonary nocardiosis also involve infections in areas outside the lungs. which usually has spread hematogenously from an asymptomatic or healed pulmonary site. Extrapulmonary local extension may also occur from the lungs, resulting in purulent pericarditis, mediastinitis, and/or the superior vena cava syndrome. Other common sites of nocardial dissemination include skin, subcutaneous tissues, and the central nervous system.

Primary Cutaneous Disease

primary cutaneous nocardiosis is usually an infection in immunocompetent hosts. Primary cutaneous infections usually presents as lymphocutaneous infection, superficial cellulitis, or localized abscess and usually involve the face in children or the lower extremities in adults. is a late-stage infection and is characterized by a chronic, localized, slowly progressive, and often painless subcutaneous and bone disease usually involving the foot. Most lesions have been present for six months or longer before diagnosis, subcutaneous nodules, destructive granulomata, and formation of intermittent fistulas, with production of pus, and granules of various sizes and colors are characteristic features of these infections. infection is typically marked by the presence of a primary pyodermatous lesion (ulceration with purulence), often with areas of chronic drainage and crusting. The organism invades more deeply to involve the lymphatic system and progresses to the formation of lymphatic abscesses. Superficial cutaneous nocardiosis is the least serious of the cutaneous infections. This form of nocardiosis usually occurs in an immunocompetent individual 1 to 3 weeks following some type of local trauma with subsequent environmental contamination of the wound.

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Antifungal Agents

An antifungal agent is a substance that kills fungi or inhibits its growth. There are many types of antifungal agents used to treat a range of illnesses and conditions caused by fungi. Some of these agents are topical while others are meant to be ingested. Many antifungal agents can be purchased over the counter for use without a doctor's guidance, but some are available only by prescription.

One type of antifungal agent is called a **systemic antifungal**. This type is usually taken orally, though doctors sometime administer them intravenously, which means through a vein. Some commonly prescribed antifungal agents are fluconazole, ketoconazole, and terbinafine. **Topical agents** are meant to be applied to the skin or nails, but some can be placed into the body's cavities. There are three primary categories of topical antifungal agents: azoles, allylamines and benzylamines, and polyenes. Each category works to destroy fungi in a slightly different way.

Polyenes kill fungi cells. Commonly used types of polyenes include Nystatin and Amphotericin B. This type of topical antifungal agent was one of the first to be developed. The polyenes bind to ergosterol present within the fungal cell wall membrane.This process disrupts cell wall permeability by forming oligodendromes functioning as pores with the subsequent efflux of potassium and intracellular molecules causing fungal death There is also evidence that Amphotericin B.(AMB) acts as a proinflammatory agent and further serves to stimulate innate host immunity. When AMB resistance occurs, it is generally attributed to reductions in ergosterol biosynthesis or the synthesis of alternative sterols with a reduced affinity for AMB. Resistance to AMB is common in Aspergillus terreus, Scedosporium apiospermum, Scedosporium prolificans, Trichosporon spp, and Candida lusitaniae. Resistance has been reported with several other species, however Before the development of alternative agents, AMB was the recommended first-line agent for invasive candidal infections. AMB in combination with flucytosine remains the drug of choice in the treatment of cryptococcal meningitis and in most cases a lipid formulation is preferred because of the decreased incidence of nephrotoxicity. 5 Severe infection caused by the endemic mycoses (ie, histoplasmosis, coccidioidomycosis, blastomycosis, and sporotrichosis) should be treated with an AMB formulation. Histoplasmosis remains the only infection for which a lipid formulation of AMB (L-AMB) has demonstrated greater efficacy than the conventional form.

Azoles are another type of topical antifungal agent. Azole antifungal drugs inhibit the enzyme lanosterol 14 α-demethylase; the enzyme necessary to convert lanosterol to ergosterol. Depletion of ergosterol in fungal membrane disrupts the structure and many functions of fungal membrane leading to inhibition of fungal growth (Fig. 1). This enzyme also plays an important role in cholesterol synthesis in mammals. When azoles are present in therapeutic concentrations, their antifungal efficacy is attributed to their greater affinity for fungal P-450DM than for the mammalian enzyme. Exposure of fungi to an azole causes depletion of ergosterol and accumulation of 14α-methylated sterols. This interferes with the "bulk" functions of ergosterol in fungal membranes and disrupts both the structure of the membrane and several of its functions such as nutrient transport and chitin synthesis. The net effect is to inhibit fungal growth. Ergosterol also has a hormone-like ("sparking") function in fungal cells, which stimulates growth and proliferation. This function may be disrupted when ergosterol depletion is virtually complete (>99%). This class of agents has demonstrated species- and strain-dependent fungistatic or fungicidal activity in vitro and the area under the curve (AUC) to MIC ratio is the primary predictor of drug efficacy.. Clotrimazole, ketoconazole, miconazole, and oxiconazole are among the commonly prescribed azoles.

Allylamines and benzylamines are another class of topical antifungal agents. These topical agents act in a similar manner to azoles, interfering with ergosterol. They interfere with this substance earlier on in its production process, however. As such, they are helpful for both inhibiting growth and killing fungi. Among the commonly used types of antifungal agents in this class are butenafine, naftifine, and terbinafine.

As with other types of medications, antifungal agents may cause side effects, regardless of whether they are used in over-the-counter or prescription form. Among the side effects possible with topical agents are burning, itching, minor pain, and rash where the antifungal agent is applied. Systemic agents are typically only available by prescription and may cause stomach upset, hives, rashes, itching, sight and taste disturbances, and headaches.

Most people who use antifungal medications do not experience side effects. Systemic antifungal agents can cause severe side effects in some people, however. For example, some of these drugs can damage the liver and the kidneys. They may even damage the heart and diminish a person's white blood cell count, possibly making it harder for him to fight off infections. For these reasons, doctors often monitor their patients' health while these medications are in use.

With the advent of the polyenes, azoles, and Allylamines and benzylamines, previously fatal infections can now be treated. However, as modern medicine continues to extend life through aggressive therapy of other life-threatening diseases such as cancer, there is an increasing population at risk for opportunistic fungal infections. Such patients represent a special challenge because they often are left with little host immune function. Therefore, chemotherapeutic agents should be fungicidal and not just fungistatic. The search continues for fungicidal agents that are nontoxic to the host. Research is also directed toward immunomodulating agents that can reverse the defects of native host immunity.

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Microbiological Testing in Food Industries

1. Traditional Methods for Detection of Food-borne Pathogens

Microbiological examination of foods and food ingredients helps to assess safety to consumers, stability or shelf life under normal storage conditions, and the level of sanitation used during processing. Thus, routine examination of foods to detect selected pathogens is necessary. Most analyses look for indicator organisms, which are more rapidly enumerated. The three basic categories of tests in microbiology include presence–absence tests, enumeration tests, and identification or characterization tests. Pioneers of microbiology developed the fundamental methods traditionally used for many of these tests.

1.1 Presence–Absence Tests

Conventional Plate Count

The most important information used to assess the quality, spoilage, and safety of foods and to determine potential implications of foodborne pathogens is determination of total viable cell counts in food, water, food–contact surfaces, and air in food plants. The Aerobic Plate Count (APC), also known as the Aerobic Colony Count (ACC), Heterotrophic Plate Count (HPC), Total Plate Count (TPC), or standard plate count, estimates the number of total viable aerobic bacteria per gram or milliliter of product. Detailed procedures for determining the APC of foods have been developed by the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA) (Maturin and Peeler, 2001).

Fungi are ubiquitous organisms that often are associated with the spoilage and biodeterioration of a large variety of foods and feedstuffs (Bleve et al., 2003). Prior to analysis, it should be assumed that xerophilic molds and osmophilic yeast are organisms that prefer reduced water for growth. Spread plating is considered superior to the pour-plate method, since surface plating results in more uniform growth and makes colony isolation easier (Tournas et al., 2000). Molds that have been stressed should be enumerated by a surface spread plate technique, which provides maximal exposure of the cells to atmospheric oxygen and avoids heat stress from molten agar. Pour plates may be used when yeast or nonstressed mold cells are being detected (Douey and Wilson, 2004). Osmophilic yeast may be incubated for up to 7 days. Plates are examined on the third day of incubation, and if mold or yeast colonies are numerous, these are counted and then counted again on the fifth day, if possible (Douey and Wilson, 2004).

1.2 Enumeration Tests

Most Probable Number

The most probable number (MPN) technique is a widely used quantification method. An MPN is estimated from responses where results are reported as positive or negative in one or more decimal dilutions of the sample (Peeler et al., 1992). Thus, unlike the aerobic plate count, the MPN does not provide a direct measure of the bacterial count.

Frequently, the composition of many food products makes it difficult to use standard plate procedures, particularly when the microbial concentration of the sample is less than 10 CFU/g. At these low concentrations, the MPN technique gives more accurate counts than the plate count method for bacterial populations.

1.3 Identification or Confirmative Test

For Coliforms: One loop of culture from each positive lauryl tryptose tube is transferred to a tube of Brilliant Green Lactose Bile (BGLB) broth with a fermentation vial. Avoid transferring the pellicle (Christensen et al., 2002). Incubate BGLB tubes at 35?C and examine for gas production at 48 ± 2 h.

For E. coli, gently shake each gas-positive EC broth tube or fluorescing EC-MUG broth tube and streak a loopful of the culture onto an LEMB or Endo agar plate. Plates are incubated at 35?C for 18 to 24 h. Nonmucoid, nucleated, dark-center colonies with or without a metallic sheen are indicative of *E. coli* (Christensen et al., 2002).

For Yeast and Molds microscopic examination after staining with crystal violet may be necessary to distinguish yeast colonies from some bacterial colonies that may resemble yeast. Microscopically, yeast cells are significantly larger than bacteria and some cells may be budding (Douey and Wilson, 2004).

2. Rapid Methods for Food-borne Bacterial Enumeration and Pathogen Detection

Foods are routinely tested with the objectives of establishing the absence of specific pathogens or toxins, to ensure food safety, and to test for total microbial load or for indicators to monitor the sanitary quality of foods. These fundamental objectives have long been achieved with traditional microbiological methods, which are labor, time, and material-intensive. Hence, rapid methods have had a major impact, as evidenced by the vast numbers of papers that describe the use of rapid methods in food testing.

RAPID PATHOGEN TESTING METHODS

Rapid pathogen testing may be divided into identification and detection methods. Bacteria may be identified by many attributes, including fatty acid and carbon oxidation profiles, but most still rely on biochemical analyses, which are labor- and media-intensive procedures. Miniaturized biochemical identification kits, which have been in use for years, have greatly simplified this process, but identification assays are becoming even more user-friendly, as many are now automated.

The use of **specialized substrates** or media for presumptive identification of bacteria is another area that has seen a lot of changes. The use of special substrates became popular with the fluorogenic substrate 4-methylumbelliferone-β-d-glucuronide (MUG) for identifying *E. coli* based on β-glucuronidase activity (Feng and Hartman, 1982). These fluorogenic and chromogenic substrates, however, continue to be used in rapid enumeration tests for indicators.

Antibody-based assays comprise the majority of rapid pathogen detection kits and they use various assay formats. Simple latex agglutination tests that use antibody-bound colored latex beads to serotype pure bacterial cultures have become very popular. Used initially for serotyping *E. coli* O157:H7 isolates from foods, latex assays are now available for many pathogens, including other enterohemorrhagic *E. coli* serotypes that are increasingly causing foodborne illness worldwide (Brooks et al., 2005).

Immunomagnetic separation (IMS) is another antibody-based format that has undergone major advancements, as many are

realizing that the selective antibody capture of target by IMS can often improve the sensitivity of other assays (Benoit and Donahue, 2003). Hence, the diversity of antibodies coupled to beads has increased, and automated IMS assays have also become available.

Pathogen detection using the antibody **Enzyme–Linked Immunosorbent Assay (ELISA)** format continues to exist, but few manual tests are being introduced. Antibodies are also being used increasingly in biosensors, which have biological components (antibodies and ligands) that are coupled with sensitive physicochemical transducer to measure specific biological interactions. Biosensors can simultaneously detect multiple targets (Taitt et al., 2004) and are very fast and sensitive in the detection of bacterial cultures in solutions, but their efficiency in food testing can be variable and is still being explored (Alocilja and Radke, 2003).

DNA-based pathogen detection assay formats consist of DNA probes (Olson, 2002), cloned phages (Favrin et al., 2001), and polymerase chain reaction (PCR). Until recently, most PCR assays, however, used manual gel-based detection that was labor-intensive, hence not very popular. As a result, DNA-based rapid pathogen methods formed only a small percentage of commercially available kits. Advances in genomics (Abee et al., 2004), however, introduced such technologies as real-time PCR (RT-PCR) (Exner, 2005) and DNA chips (microarrays) (Call, 2005), which caused a resurgence in DNA-based assays. As a result, RT-PCR has surpassed other DNA formats, and many new assays for pathogens have been introduced. These assays use fast amplification and a variety of detection technologies (Kubista and Zoric, 2005) for real-time data monitoring that will give results in an hour.

Rapid Enumeration Methods for Foods

A wide range of both direct and indirect rapid enumeration methods have been developed for testing for total and indicator bacteria in foods. They are divided into manual and automated tests.

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Rapid Enumeration Assays for Food Samples

Type	Assay	Principle/Description	Applications
Manual	Petrifilm Sanitakun	Dual-layer film coated with nutrients and gelling agents soluble in cold water	Food, water, environmental
	Iso-Grid	Membrane filtration using hydrophobic grid membranes that are placed on selective agar for enumeration by MPN	Wide range of foods, beverages, ingredients, water
	Easygel	Special petri dish coated with gelling agents and nutrients	Wide range of foods, beverages
	Simplate Quanti-Disc	Multienzyme technology Correlate enzyme activity with the presence and number of viable organisms in foods	Wide variety of foods (Simplate), water (Quanti-Disc)
	ColiTrak, ColiTrak Plus (fecal coliform)	MPN assay utilizing LST and MUG (fecal coliform)	Food, water, environmental
	Millipore's Samplers	Ready-to-use plastic paddle with nutrients, membrane filter for sampling and incubation	Water
Automated	Spiral, Wasp II Spiral Plater	Precise delivery of 1 : 1 to 1 : 10,000 sample dilution in a spiral pattern on each plate	Food, water, environmental
	Tempo	Automated MPN: coliforms, *E. coli*, and Enterobacteriaceae	Poultry, egg, meat, meat products
	Bactometer, Rabbit Impedance Detection System, Bac Trac	Impedance	Food, water, environmental
	Malthus	Conductance	Food, water, environmental
	BacT/Alert Automated Microbial Detection System	Colorimetric sensor and reflected light to monitor cell presence by production of CO_2	Food, beverages
	ChemScan RDI System	Flow cytometry (fluorescent cell labeling and laser scan)	Pharmaceutical, personal care, food, beverages
	D-Count	Flow cytometry (fluorescent cell labeling, laser excitation, and digital processing)	Pharmaceutical, personal care, food, beverages
	Milliflex Rapid Microbiology Detection System	ATP bioluminescence	Water and filterable samples

Microxpress introduce a new Accessory product Nyflock (Nylon flocked Swab). Nylon Flocked Swab is used in a wide variety of applications.

Clinical Requirement:
Nylon Flocked Swabs is used for virology culture, direct immunofluorescence assay (DFA) testing for H1N1 influenza, Collection of bacteriology samples from different areas like wound, mouth, ear and vaginal samples, Collection of DNA Samples for Forensic Applications.

Pharmaceutical Requirement:
Nylon Flocked Swabs is used for environmental sampling and sterility testing of different areas.

Nylon flocked Swab gives recovery in terms of bacterial CFU 3 times greater than traditional fiber swab (20% to 60%). It gives better recovery in terms of-

Better Sample Collection
Liquid secretions are rapidly absorbed between the perpendicular fibers by capillary action.

Cellular material is collected very easily and efficiently because the velvet brush-like texture of the flocked swab is more effective at dislodging and removing cells.

Entire Sample Released
The entire sample is instantaneously and automatically released the moment the flocked swab is mixed with assays reagents or placed in a buffer solution or transport medium.

Feature and benefits of Nylon Flocked swab

	Feature	Benefit
1.	**Ergonomic**(designing equipment as per human convenience) and **Anatomic Design** (Related to the structure)	For improved patient comfort and efficiency in specimen collection.
2	**Rapid Automatic Elution** (The removal or separation of one material from another)	Instant and spontaneous release into liquid Media
3	**Improved Sample Collection**	Velvet brush-like texture rapidly and efficiently dislodges cells and collects liquid by capillarity.
4	**Quantitative Volume Transfer**	Measurable and consistent uptake and transfer from patient to test tube

Nyflock **Pack Sizes**: 100 No. (Individually Pack)

BioShields Presents Nusept

Composition - 1% w/v Poly (hexamethylene biguanide) hydrochloride, Perfume, Fast green FCF as color.

Description: NUSEPT™ is a new generation, powerful, non stinging, safe, highly effective and resistance-free microbicidal antiseptic solution. NUSEPT™ is an ideal antiseptic for use in medical settings. The main active ingredient of NUSEPT™ is poly (hexamethylenebiguanide) hydrochloride (PHMB). PHMB is a polymeric biguanide. There is no evidence that PHMB susceptibility is affected by the induction or hyper expression of multi-drug efflux pumps, neither there have been any reports of acquired resistance towards this agent.

ACTIVITY : Broad spectrum: Bactericidal, Fungicidal & Virucidal.

CONTACT TIME :1 min (undiluted & 10% v/v solution), 5 min (5% v/v solution), 10 min (2.5% v/v solution).

APPLICATIONS :
Medical: In Hospitals, Nursing homes, Medical colleges, Pathological laboratories for Inter-operative irrigation. Pre & post surgery skin and mucous membrane disinfection. Post-operative dressings. Surgical & non-surgical wound dressings. Surgical Bath/Sitz bath. Routine antisepsis during minor incisions, catheterization, scopy etc. First aid. Surface disinfection.
Industrial: In Pharmaceutical industry, Food & beverage industry, Hotel industry etc. General surface disinfection. Eliminating biofilms.

USAGE DIRECTIONS :

Surgical, postoperative, non surgical dressings	–	Use undiluted
Pre & post surgery, skin cleaning & disinfection	–	Use undiluted
Surgical/Sitz bath	–	Add 50 ml of NUSEPT™ in 1L of water & use
Antisepsis during minor incisions, catheterization,	–	Use undiluted scopy, first aid, bites, cuts stings etc
Midwifery, nursery & sickroom	–	Use undiluted
General surface disinfection	–	Add 100 ml of NUSEPT™ in 1L of water and gently mop the floor or surfaces

Highlights of the coming issue

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