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Editorial

Contents

Our JHS team is thankful to all our readers for their ever increasing appreciation that has served as a reward & motivation for us. Here's another issue of JHS coming your way……..

Our **Mini Review** section talks about "The applications of chromogenic media in clinical microbiology" which has been made commercially available providing useful tools for diagnostic clinical microbiology. By the inclusion of chromogenic enzyme substrates targeting microbial enzymes, such media are able to target pathogens with high specificity. The inclusion of multiple chromogenic substrates into culture media facilitates the differentiation of polymicrobial cultures, thus allowing for the development of improved media for diagnosis of urinary tract infections and media for the enhanced discrimination of yeasts.

This time's **Current Trends** section focuses on " Automated Endoscope reprocessors" in which we discussed about pros and cons of manual endoscope cleaning and compliance of EN ISO 15883 standard for Automated Endoscope Reprocessors. AER provides a workflow for endoscope reprocessing and documentation, which is an advantage compared to manual endoscope cleaning.

Our **In Profile** scientist for the month is Mr. Natteri Veeraraghavan who was an Indian physician, microbiologist and medical researcher, known for his contributions to the understanding of diseases like rabies, tuberculosis and leprosy. He was a former director of the Pasteur Institute of India, Coonoor and the chairman of the World Health Organization International Reference Center on Rabies. He was honoured by the Government of India in 1967, with the award of Padma Shri, the fourth highest Indian civilian award for his contributions to the society.

This months **Bug of the Month** is ***Burkholderia cepacia* complex (BCC)**, or simply ***Burkholderia cepacia***, which is a group of catalase-producing, lactosenonfermenting, Gram-negative bacteria composed of at least 20 different species, including *B. cepacia, B. multivorans, B.cenocepacia, B. vietnamiensis, B. stabilis, B. ambifaria, B.dolosa, B. anthina, B. pyrrocinia* and *B. ubonensis*. *B. cepacia* is an opportunistic human pathogen that most often causes pneumonia in immunocompromised individuals with underlying lung disease.

Under **Did you Know** segment you can read about " Babies born by C-section have more potentially infectious bacteria in their guts". Along with the birth mode itself, antibiotics delivered during birth also shape which bacteria set up shop in an infant's gut, the study suggests. In many places, mothers who undergo C-sections receive antibiotics to prevent infections. Along with removing potential threats, these drugs can kill helpful bacteria.

In **Best Practices** you can read about how Managing inflammation, moisture, edge, biofilms on wounds along with resistance bacterial invasion can be a challenge to clinicians. Systemic use of antibiotics is not enough to treat chronic wounds and understanding the correct use of antimicrobial therapy is crucial not only in preventing wound infection but also in promoting wound healing for the patient. This is a continuation of the earlier issue.....

All work & no play makes Jack a dull boy! We don't forget that ever. Each issue comes with its own bouquet of jokes, so enjoy……

So go on, enjoy reading & don't forget to give us your valuable inputs & feedback.

The application of chromogenic media in clinical microbiology

Since 1990, a wide range of chromogenic culture media has been made commercially available providing useful tools for diagnostic clinical microbiology. By the inclusion of chromogenic enzyme substrates targeting microbial enzymes, such media are able to target pathogens with high specificity. Examples of target pathogens include *Staphylococcus aureus, Streptococcus agalactiae, Salmonella* spp. and *Candida* spp. The inclusion of multiple chromogenic substrates into culture media facilitates the differentiation of polymicrobial cultures, thus allowing for the development of improved media for diagnosis of urinary tract infections and media for the enhanced discrimination of yeasts. The purpose of this review is to provide some insight into how such media work and appraise their utility in routine clinical diagnostics, in comparison with conventional media.

Introduction

The traditional approach to the detection of pathogenic bacteria in pathological specimens has typically involved the inoculation of one or more general purpose culture media such as Blood agar. Such media allow the growth of a wide range of bacteria, and suspect pathogens are detected on the basis of their colonial appearance (e.g. pigmentation, morphology, haemolysis). Such colonial characteristics rarely permit more than a presumptive identification and biochemical and / or serological tests are required for definitive identification. This approach frequently necessitates the testing of commensal bacteria that may resemble pathogens. These conventional methods are time consuming and in spite of low cost often have difficulties for microbiologist. In some bacteria like *Salmonella* it is necessary to take steps of pre-enrichment, enrichment and selective plating. Fortunately discovering rapid detecting methods have solved most of these problems. These methods have high accuracy, rapid and with the help of them in shortest period of time the microorganism can be identified. About modern techniques that applied in recent years for this purpose we can hint to genetically, physical and immunological method. Each one of these technique equipped lab and experienced technician are necessary and have expensive performs cost beside each of them have their advantage and disadvantage.

Lately using chromogenic media is one of the rapid diagnostic methods that introduced as appropriate alternative for conventional method in developed countries and applying these sensitive, accurate and specific methods in diagnosis process is a turning point in analytical microbiology and considered as powerful tools. Although chromogenic culture media first time introduced in 1979 by Rambach but it officially offered and produced since 1991.

Over the last 30 years, a range of chromogenic media has been developed that are designed to target pathogens with high specificity. Such media exploit enzyme substrates that release coloured dyes upon hydrolysis, thus resulting in pathogens forming coloured colonies that can easily be differentiated from commensal flora. Ideally, commensal bacteria should either be inhibited completely by selective agents or form colourless colonies thus allowing pathogens to 'stand out' against background flora. It is rare, however, for a pathogen to exclusively produce any particular enzyme and it is customary for a second complementary chromogenic substrate to be incorporated causing some commensal bacteria to generate either a second colour or a combination of both colours, thereby providing differentiation from the target pathogen.

Chromogenic substrates

A wide range of coloured molecules or 'chromogens' has been derivatized to produce enzyme substrates that release the coloured product following hydrolysis by a specific enzyme. For the purposes of this review, discussion will be restricted to those that have been applied in agar media for the detection of pathogenic bacteria and fungi. An ideal chromogenic substrate should be hydrolysed to release a coloured product that remains highly localized on microbial colonies. This allows clear differentiation of microbes producing the target enzyme from those that do not. This is especially important when attempting to detect specific pathogens within polymicrobial cultures.

The substrate and products of hydrolysis should be noninhibitory to microbial growth. Most commercially available chromogenic media have exploited indoxylic substrates. Indoxyl, and its halogenated derivatives, can be derivatized to form a range of esters. Release of indoxyl via hydrolysis by a specific bacterial enzyme results in the formation of brightly coloured indigo dye. This is due to spontaneous dimerization of indoxyl molecules in the presence of oxygen. Halogenation of the indoxyl molecule has a dramatic effect on the colour and intensity of this chromogen. For example, 5-bromo-4-chloro-indoxyl forms a bright green/blue dye whereas 5-bromo-6-chloro-indoxyl forms a magenta dye.

Indoxylic glycosides including glucoside, galactoside and glucuronide derivatives are widely used because of their high sensitivity, low toxicity and availability from a number of commercial sources. Other core molecules have been exploited in the design of chromogenic substrates. One particularly useful group of molecules comprises metal chelators. Esculin is a naturally occurring glycoside and a useful substrate for detection of b-glucosidase. Hydrolysis of esculin leads to release of 6,7-dihydroxycoumarin (esculetin), which binds iron to form a brown / black complex. The application of esculin in agar media is limited by diffusion of the coloured complex. A modified esculetin derivative, 3,4-cyclohexenoesculetin, was synthesized and derivatized to form glycosides that have been utilized in agar media. Hydrolysis of these substrates releases 3,4-

cyclohexenoesculetin, which, in the presence of iron, forms a black chelate, which remains highly localized within bacterial colonies and does not diffuse through agar.

8-Hydroxyquinoline is another metal chelator, which has been derivatized to form glycosides that have been exploited in agar media. When released by hydrolysis, 8-hydroxyquinoline binds with iron to form a brown/black chelate. Toxicity of this chelate towards Gram-positive bacteria has precluded the more widespread application of such substrates. Other chelation-based substrates applicable to agar media include those based on alizarin and 3', 4'-dihydroxyflavone although these have not yet been applied in commercially available chromogenic media.

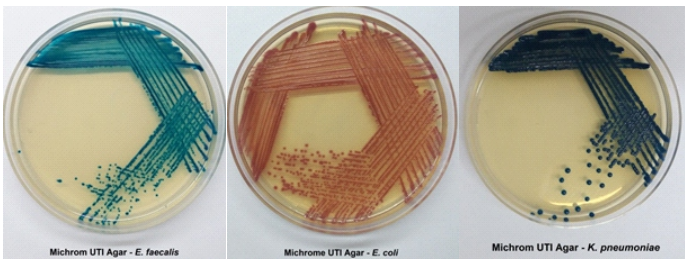
The synthesis of a series of extended phenolics has been reported and closely related compounds have since formed the basis of chromogenic substrates for the detection of glycosidases and esterases. These have been applied for the detection of *Candida* spp. and *Salmonella* spp. The attributes of a range of chromogens derivatized as substrates for β-glucosidase have recently been compared and their chemical structures illustrated.

Detection of urinary tract pathogens

Kilian and Bulow (1979) described a chromogenic medium that employed a substrate for β-glucuronidase, thus allowing the direct detection of the commonest pathogen of the urinary tract, *Escherichia coli*. In a large study using 9247 urine samples, the authors reported that 94% of *E. coli* strains could be identified by their coloured appearance alone and no *E. coli* strains were misidentified. When compared with the use of conventional media (Blood and MacConkey agars), the authors reported a cost reduction of 46% for media, and a 67% reduction in the time required for processing samples. In this study, as in most others, *E. coli* accounted for the majority (58%) of isolates with other *Enterobacteriaceae* (24%) and enterococci (11%) also isolated frequently. The cost-effectiveness of using chromogenic media for urine culture because of decreased labour time and lower usage of reagents has been demonstrated by others.

As illustrated above, amongst the *Enterobacteriaceae*, *E. coli* are the only β-glucuronidase producers that are likely to be found in urine samples. In addition to a β-glucuronidase substrate, this medium employs a substrate for detection of β-glucosidase activity in enterococci and the *Klebsiella/Enterobacter/Serratia* group, as well as tryptophan for demonstration of tryptophan deaminase in the Proteus- Providencia-Morganella (PPM) group. The medium allowed direct identification of the commonest urinary tract pathogens on the basis of colony colour without the need for laborious confirmatory tests. Over recent years a range of media, based on similar principles, has been made commercially available. These media generally employ either β-glucuronidase or β-galactosidase for the identification of *E. coli*, tryptophan for the identification of the PPM group and a β-glucosidase substrate for the detection of Enterococci. The use of a β-galactosidase substrate reduces the specificity of identification for *E. coli* and some species, e.g. *Citrobacter freundii* may be misidentified as *E. coli* unless supplementary biochemical tests (e.g. for indole production) are employed.

Several authors have compared the performance of these chromogenic media against each other and against conventional agars. Aspevall et al. (2002) compared four chromogenic media along with cystine lactose electrolyte deficient (CLED) agar and MacConkey agars with 1200 urine samples. They concluded that the chromogenic media were slightly better than the conventional media because of their superior ability to differentiate mixed cultures. This point has been supported by other studies. A wide range of complementary tests has been recommended for use with chromogenic media including tests for indole production, pyrrolidonyl peptidase, urease and lysine or ornithine decarboxylases. Such tests are used either to increase the specificity of *E. coli* identification, or to broaden the range of species that may be identified with the assistance of chromogenic media.

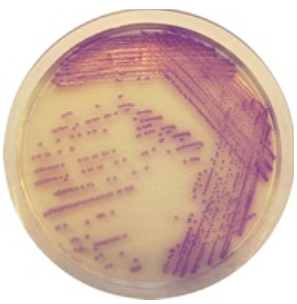


Detection of *Staphylococcus aureus*

Isolation of *Staphylococcus aureus* is usually achieved by culture of specimens on general purpose media, such as blood agar, and subsequent identification of suspect colonies using biochemical and/or serological tests. Most commonly this involves testing colonies of staphylococci for agglutination with sensitized latex particles to detect bound coagulase, protein A and/or specific capsular antigens. Chromogenic media have been designed for the isolation and detection of *Staphylococcus aureus* with some success. Chromogenic media for *Staphylococcus aureus* is a selective agar medium that employs a combination of chromogenic enzyme substrates. *Staphylococcus aureus* strains grow as mauve colonies on this medium whereas most other staphylococci produce white, or occasionally, blue colonies. A number of complementary tests may be used with chromogenic media, for example coagulase detection to confirm identification or detection of methicillin resistance using latex agglutination.

Detection of Methicillin Resistant *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a nosocomial pathogen of world-wide importance and an increasingly frequent cause of community-acquired infection. A wide range of culture methods has evolved for the detection of MRSA. Such methods have traditionally employed selective media supplemented with oxacillin or methicillin to suppress the growth of methicillin-sensitive *Staphylococcus aureus* (MSSA). Mannitol salt agar supplemented with oxacillin is widely used but has shown limited sensitivity and specificity in some studies. A modified version of mannitol salt agar is Oxacillin Resistance Screening Agar base (ORSAB), which is more selective due to the presence of lithium chloride and polymyxin, and contains aniline blue as a pH indicator. Some studies have revealed similar limitations regarding sensitivity and specificity.



Ciprofloxacin is a useful agent for the suppression of MSSA strains and has been used successfully to supplement Baird-Parker medium

and mannitol broth. These methods are limited; however, as they cannot detect ciprofloxacin sensitive MRSA which may occur in some areas.

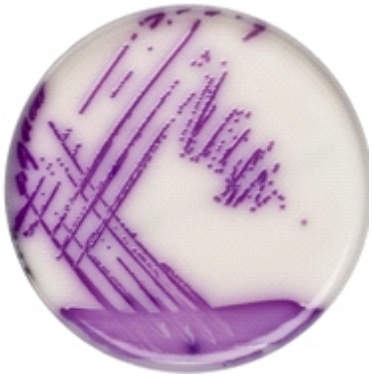
Detection of *Salmonella*

A range of chromogenic media has been developed for the detection of *Salmonella* spp. in stool samples. Most of these media have been designed using similar principles. Rambach agar and Salmonella Detection and Identification medium (SM-ID) were the first media of this type. In common with several subsequently developed media, Rambach agar employs a chromogenic substrate for β-galactosidase causing common commensals such as *E. coli* to generate blue colonies. The vast majority of *Salmonella* spp. isolated from humans do not produce β-galactosidase and are highlighted instead as red colonies because of their ability to acidify neutral red by fermentation of propylene glycol (Rambach 1990). SM-ID also incorporates a β-galactosidase substrate and glucuronic acid, which is fermented by *Salmonella* spp.

Alpha–beta chromogenic medium (ABC medium) also utilises a β-galactosidase substrate and *Salmonella* are visualized by their ability to hydrolyse a second chromogenic substrate for alpha galactosidase. When compared with conventional agars, the high specificity of ABC medium has been shown to offer a highly. cost-effective means of detecting *Salmonella* spp. In a large study with 2409 stool samples, Nye et al. (2002) confirmed the high specificity of ABC medium but reported a low sensitivity as a primary plating medium, when compared with conventional agars, due to overgrowth of commensal flora. Perry et al. (2002) evaluated ABC medium in a multicentre study on three continents and found that 45% of *Salmonella* isolates from Burkina Faso, Africa, failed to produce alpha galactosidase and would therefore not be routinely detected. This emphasizes the need to validate the performance of chromogenic media in different geographical locations.

Few species of *Enterobacteriaceae* apart from *Salmonella* spp. are able to cleave fatty acid esters of 7–10 carbon atoms (e.g. octanoate derivatives). In recent years a range of media has been manufactured that rely on the detection of such activity in *Salmonella* spp., using chromogenic esters. Other species that produce such esterases, such as *Pseudomonas* spp. and yeasts may be inhibited by incorporation of selective agents such as cefsulodin and amphotericin, respectively.

Chromogenic media offer a much higher degree of specificity than conventional media which are based on absence of lactose fermentation within *Salmonella* and/or their ability to generate hydrogen sulphide. However, some reports have shown that conventional media offer a higher degree of sensitivity particularly when specimens are plated directly onto agar media. This is largely explained by findings showing that some chromogenic media for *Salmonella* spp. exhibit very poor selectivity against common commensals such as *E. coli*. An innovative approach to the inhibition of *Enterobacteriaceae* other than *Salmonella* involves the application of enzyme substrates, which release a toxic product upon hydrolysis. This approach permits the rationale design of inhibitors selecting for bacteria that lack a particular enzyme or show a reduced uptake of the 'suicide substrate'. Such an approach may increase the selectivity of chromogenic agar, as demonstrated by the inclusion of alafosfalin in ABC medium. Following enrichment in selenite broth, *Salmonella* spp. predominate and their detection can be conveniently confirmed with high specificity using chromogenic media. Direct inoculation of solid media remains necessary for primary diagnosis in order to facilitate early detection of *Salmonella* and because of the susceptibility of some strains to selective enrichment procedures. A further limitation of chromogenic media is that other enteric pathogens, e.g. *Shigella* spp. are not detected and complementary media are therefore required for routine examination of stool samples.



Detection of *Escherichia coli* O157

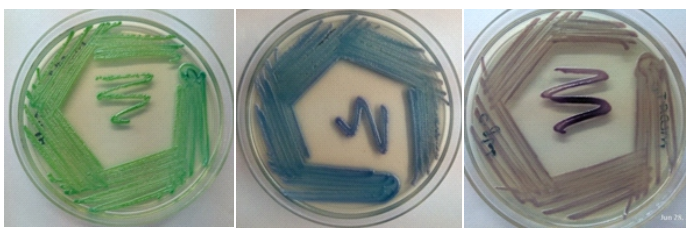
Escherichia coli O157: H7 is the predominant clone associated with outbreaks of haemorrhagic colitis and haemolytic uraemic syndrome. Although outbreaks are uncommon, symptoms may be severe, leading to death, and in the UK this has led the Subcommittee of the PHLS Advisory Committee on Gastrointestinal Infections (2000) to recommend culture of all diarrheal stools for *E. coli* O157. Several chromogenic media have been applied to the detection of *E. coli* O157 from stool samples. Most are based on similar principles; relying on nonfermentation of sorbitol and / or rhamnose and lack of β-glucuronidase activity in the commonest serotype, *E. coli* O157: H7. These biochemical markers, in association with selective agents, help differentiate nonpathogenic *E. coli* and other commensals from most strains of *E. coli* O157: H7. A second chromogenic substrate (e.g. for α or β-galactosidase) may be used to highlight the presence of *E. coli* O157 amongst nonreactive background flora.

Detection and differentiation of yeasts

A range of chromogenic media is available for detection of yeasts. Such media have a superior ability to differentiate mixtures of different species when compared with traditional media such as Sabouraud agar. A common principle among these media is the inclusion of a chromogenic substrate for β-hexosaminidase thus allowing the differentiation and identification of the most frequent and clinically important species, *Candida albicans*. Freydie`re et al. (2001) have reported that detection of *C. albicans* via β-hexosaminidase activity is as sensitive, more specific, less subjective and far more rapid than the conventional germ tube test. For this reason, and following a detailed cost analysis, the authors concluded that chromogenic media for isolation of yeasts are economically advantageous compared with conventional media. This finding has been supported by others.

The use of Michrom Candida Agar, employs a combination of chromogenic substrates to give enhanced differentiation of yeast species. As well as the differentiation of *C. albicans* as green colonies, this medium allows for identification of *Candida tropicalis* as blue colonies. Other species form either pink or white colonies. *Candida krusei* may be recognized by the formation of characteristic pink flat colonies although some less

commonly encountered species such as *Candida inconspicua* have been shown to produce a similar colonial appearance. It is often claimed that a range of species may be specifically identified using chromogenic media when factors such as colony size and morphology and colour intensity are taken into consideration, however, this can be highly subjective and require a lot of experience. Limitations of chromogenic media for yeasts include the lack of a specific chromogenic marker for *Candida glabrata* which is commonly found to be the non *albicans* species most frequently isolated from clinical samples. A further limitation is the difficulty in distinguishing *Candida dubliniensis* from *C. albicans* as both demonstrate β-hexosaminidase activity. This hybrid medium stimulates enhanced chlamydosporulation by *C. dubliniensis* and the consequent formation of rough colonies that are distinguishable from those of *C. albicans*. Other workers have employed supplementary biochemical or serological tests to be used as adjuncts to chromogenic agars. For example, latex agglutination tests can be used to specifically identify colonies of *C. krusei* and *C. dubliniensis* on chromogenic agars. The utility of a rapid test for trehalose assimilation to allow rapid identification of *C. glabrata* is demonstrated.



(1) (2) (3)

Michrom Candida agar: 1) *Candida albicans 2) Candida tropicalis 3) C. glabrata*

Detection of *Streptococcus agalactiae*

Streptococcus agalactiae (group B *streptococcus*) is a leading infectious cause of morbidity and mortality among newborns. This has led some authorities to recommend routine screening of vaginal swabs for the presence of this pathogen in pregnant women. A chromogenic medium, has recently been described for the detection of *S. agalactiae* as red colonies. In a study with 134 vaginal swabs, Chromogenic media was shown to have a higher specificity and sensitivity than blood agar for detection of *S. agalactiae*. In a larger study with 737 samples, a higher sensitivity of Chromogenic media than Granada medium or a selective blood agar for recovery of *S. agalactiae*. The authors reported an enhanced recovery of strains after prolonged incubation for 48 h and underlined the requirement of selective broth enrichment to further enhance isolation.

Conclusions and future developments

In many studies chromogenic media demonstrate a proven advantage over conventional culture media due to a superior detection rate for target pathogens or a superior differentiation of mixed cultures. Media containing chromogenic substrates are invariably more expensive than conventional media but this can be offset by a reduced need for complementary reagents and less labour time associated with the processing of culture plates and suspect pathogens. Due to these factors, the use of chromogenic media in diagnostic laboratories is increasingly widespread. It is likely in the next few years that a wider range of pathogens will be targeted to continue the rapid expansion of the range of chromogenic media. Chromogenic media are available for isolation of pathogenic *Vibrio* spp. and *Campylobacter* spp. from food samples and it is likely that such media may be evaluated and / or adapted for clinical diagnostics. The usefulness of chromogenic media for MRSA should prompt the further development of screening media for other antibiotic-resistant pathogens such as glycopeptide resistant enterococci and extended spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* and examples of such media are already available. The development of new media may depend upon the recognition and synthesis of new chromogenic substrates with diagnostic utility. Almost all commercially available media have relied upon the application of a limited range of chromogenic substrates based on detection of glycosidase, phosphatase or esterase activity. Most bacterial pathogens produce a wealth of peptidases but these have remained unexploited thus far. Recognition of useful enzyme activities and design of appropriate chromogenic substrates may lead to the development of chromogenic culture media for other bacterial pathogens such as *Legionella* spp.

In comparison with other method chromogenic media are more rapid, accurate and reliable and can utilize as alternative for conventional methods. Hence employing these media eliminate the need of subculture and further biochemical test for identification of pathogenic agent and at the shortest period of time possible, pathogenic agent can be identified. This feature especially in in disasters and military condition like maneuver and military camps have special important for preventing food and water borne outbreak.

Automated Endoscope Reprocessors (Issue III)

6.5.4 Manual Endoscope Cleaning

RECOMMENDATION

Wherever possible, EWDs that comply with EN ISO 15883 standard series should be used. If this is not possible manual reprocessing should be performed based on standard operating procedures.

ESGE and ESGENA are aware of the varying economic situations in different countries. Nevertheless, hygiene standards for patient and staff safety should have the highest priority. National guidelines emphasize the preference for automated reprocessing after manual cleaning. However, the British and Dutch guidelines clearly state that manual disinfection is no longer acceptable, except in the case of technical problems with the EWD. Ultimately, it is the responsibility of the clinical service provider to choose an effective reprocessing method in line with national laws and regulations. See Table 4 for the advantages and disadvantages of manual disinfection.

Table 4 Advantages and disadvantages of manual endoscope disinfection

Advantages	Disadvantages
▪ Easy to establish	▪ No standards/guideline available for validation of manual disinfection
▪ Moderate investments	▪ Validation difficult, however, standardization for all reprocessing steps is possible ▪ Increased risk of human error (inconsistencies, mistakes, etc) ▪ Staff exposure to process chemicals and potentially infectious material; additional precautionary measures necessary ▪ Increased workload, because staff involved in each reprocessing step ▪ In the case of reuse of disinfectant, efficacy problems to be considered ▪ Traceability and documentation activities are more time-consuming and more difficult ▪ Increased risk of recontamination, followed by increased risk of infections for patients ▪ Increased risk of health problems for staff (infection, injuries, allergies, etc.)

RECOMMENDATION

In the case of manual disinfection:

- A sufficient number of sinks of suitable size for endoscope reprocessing should be available.
- All cleaning steps should be performed prior to disinfection.
- An intermediate rinsing step is necessary between cleaning and disinfection.
- For disinfection, the endoscope should be immersed completely, and all channels should be filled completely with disinfectant.
- The manufacturer's recommendations regarding correct concentration, temperature, contact time, and number of reuse cycles (if applicable) should be followed, and this compliance should be documented, in order to ensure adequate disinfection.

If the disinfectant is a concentrated product, it must be diluted, in the correct dilution ratio, with filtered water or drinking water of defined quality. Freshly prepared disinfecting solution provides the largest safety margin. Use of the disinfecting solution for a longer period risks lowering the concentration by, for example:

- Decomposition of the active substance.
- Adsorption of active substance onto surfaces.
- Inactivation of the active substance by reaction with protein.
- Dilution of the disinfecting solution by rinse water remaining in the endoscope from the previous reprocessing step.

6.6 Final Rinsing

RECOMMENDATION

Disinfectant solution should be rinsed from the internal and external surfaces of the endoscope with sterile filtered water. National requirements regarding water quality should be followed.

RECOMMENDATION

Rinsing water should not be reused at any time.

Rinse water quality is an important issue. It must be at least of drinking water quality and should be free of pathogens such as *Pseudomonas aeruginosa*. Preferably, sterile filtered water may be used for rinsing.

Insufficient rinsing can cause severe damage to patients. Disinfectant residues on endoscope surfaces can cause severe complications such as colitis, abdominal cramps, and bloody diarrhea. This occurs mainly after manual reprocessing procedures.

Up to 50mL of solution can remain in an endoscope (depending on endoscope type) if not removed by compressed air.

6.7 Drying of Endoscopes

RECOMMENDATION

The endoscope and its components should be dried after completion of the cleaning and disinfection process. The required intensity of drying depends heavily on the intended further use of the endoscope:

- If the endoscope is to be used for the next patient examination within a short period of time, removal only of major water

residues from the endoscope channels and outer surfaces will be sufficient.
- If the endoscope is not to be reused immediately and is to be stored, the endoscope channels and outer surfaces should be dried thoroughly, in order to avoid any microorganism growth leading to recontamination.
- If the endoscope is used directly after reprocessing, it must be placed in a clean and covered transport tray.

Thorough drying of endoscope surfaces and channels is necessary to prevent any growth of waterborne microorganisms. Several outbreaks of *P. aeruginosa, K. pneumoniae, Acinetobacter spp.* and other pathogens have been caused by insufficient drying. Furthermore, biofilms and embedded microorganisms need moisture for survival.

Endoscope valves can also show contamination after reprocessing and may be the source of infections if cleaning, drying, storage, and hand hygiene are inadequate. There is an increasing trend for using detachable endoscope components as single-use products to enable full traceability and to prevent cross-infection caused by inadequately reprocessed detachable components such as valves and distal caps.

All external parts and all endoscope channels must be dried carefully with compressed air specially provided for drying.

Manual drying processes can be avoided by using EWDs with a dedicated endoscope drying function or by use of specialized endoscope storage and/or drying cabinets that comply with EN 16442 standard.

RECOMMENDATION
Flushing of endoscope channels with alcohol for drying purposes is not recommended.

In various countries the use of alcohol is banned, because of potential protein fixation risks. There is no clear evidence that flushing with alcohol is effective in either drying of endoscopes or in preventing the proliferation of waterborne bacteria.

However, attitudes to the use of alcohol for drying endoscope channels are quite diverse. Some guidelines still recommended flushing with 70%– 90% ethanol or isopropyl alcohol to facilitate the drying of endoscope channels. Updated national guidelines consequently recommend the use of drying cabinets.

6.8 Storage of Endoscopes
RECOMMENDATION
Endoscopes should be stored:
- Vertically in well-ventilated, closed cupboards or
- In purpose-designed storage cabinets with/without a drying function.

RECOMMENDATION
During storage, endoscope components such as valves and distal caps should be disconnected from the endoscope. Whenever possible, endoscope components should stay with the named endoscope as a set, to enable full traceability and to prevent cross-infection.

RECOMMENDATION
Endoscopes should never be stored wet or before decontamination has been completed as such storage supports the growth of microorganisms and biofilms.

Outbreaks connected to insufficient drying and storage were mainly reported when instructions for drying had not been followed. Storage in a controlled environment is aimed at preventing any secondary contamination.

For storage of endoscopes, suitable and well-ventilated locations, generally for vertical placement, should be selected. If nonvertical storage is chosen, special attention will be needed to ensure that no residual moisture will cause recontamination of the endoscope. Updated national guidelines consequently recommend the use of drying cabinets.

For reasons of traceability and prevention of cross-infection, endoscope components such valves or detachable distal caps stay with the endoscope, but are disconnected in order to avoid any air blockage/any moist chamber in the endoscope channels. There is a clear trend toward single use of these components.

6.8.1 Storage Cabinets With/Without A Drying Function
RECOMMENDATION
In the storage cabinets:
- Only fully cleaned and disinfected endoscopes should be stored.
- All endoscope channels should be connected using purpose-designed adapters for air ventilation purposes.
- Endoscope components (such as valves) should also be stored and dried with the endoscope that they have been used with.

RECOMMENDATION
If storage in cabinets with/without a drying function is used:
- Maximum storage duration should be consistent with the manufacturer's IFU of the cabinet and in line with local regulations.
- Regular maintenance should be performed.
- Routine microbiological surveillance should be done when the maximum storage time specified by the manufacturer has elapsed.

The European standard for endoscope storage cabinets (EN 16442) sets minimum product specifications and deals with all aspects of product type testing and performance qualification. EN 16442 specifies how storage cabinets must be designed in order to achieve a controlled environment, and to prevent recontamination risks.

A number of national guidelines recommend the use of storage cabinets.
The main performance requirements are:
- Cabinets must be able to at least keep the microbiological quality of cleaned and disinfected endoscopes unchanged during storage.
- The quality of air inside the cabinet must be specified.
- The maximum storage period for endoscopes must be determined.
- Cabinets without an endoscope drying function must have

instructions for the user on how to dry endoscopes prior to placement in the cabinets.
- If drying is part of the cabinet function, maximum drying times must be specified.
- The cabinets must be provided with suitable connectors for all compatible endoscopes.
- The connectors must assure sufficient airflow though all channels of compatible endoscopes.

6.8.2 Shelf-Life of Reprocessed Endoscopes/Expiration Of Storage

RECOMMENDATION

Local policies should be in place regarding the shelf-life of endoscopes, as the recommended shelf-life of endoscopes depends on the storage conditions, national guidelines, and the manufacturer's IFU for storage cabinets that comply with EN 16442.

The storage time of reprocessed endoscopes (shelf-life) has been the subject of debate and differing interpretations in many countries. If endoscopes are stored vertically in closed cabinets, British, Dutch and French guidelines define a time limit up till when the endoscope may be used. This time limit differs between 3 to 12 hours. If this time limit is exceeded, the whole reprocessing cycle must be repeated.

Studies with small numbers have shown contamination after 5 –7 days, and up to 14 days, identifying mainly common skin organisms rather than significant pathogens. The American multisociety guidelines and the German guidelines rated the data as not significant enough to define any maximum shelf-life. They emphasize that the shelf-life depends on the microbiological quality of the final rinse inside the EWD, the effectiveness of drying, and possibly the risk of recontamination.

In a systematic review Schmelzer et al. concluded that appropriately disinfected endoscopes can be stored for up to 7 days, if regular microbiological surveillance confirms the effectiveness of reprocessing.

Manufacturers of storage cabinets compliant with EN 16442 specify, based on type test results:
- Compatible endoscopes;
- Safe storage periods; and
- Means to validate storage conditions.

6.9. Routine Inspection

RECOMMENDATION

Visual inspections of reprocessed endoscopes should be performed after each reprocessing cycle and/or before each patient use in order to identify small cracks and wear and tear and to detect any remaining debris.

RECOMMENDATION

Routine maintenance programs offered by manufacturers must be followed.

Recent outbreaks related to ERCP suggest that it may be difficult to be detect microlesions by routine leak tests. Therefore, an additional inspection, for example with magnifying glasses, may be helpful to identify cracks and wear and tear.

This is recommended especially for complex and fragile components such as the elevator mechanism or glass lenses.

In order to prevent the consolidation of microlesions, manufacturers offer routine maintenance and exchange of components that are exposed to increased mechanical stress and wear and tear.

6.10 Sterilization of Endoscopes

RECOMMENDATION

Only if medical indications show that sterilization of flexible endoscopes may be appropriate, a low temperature sterilization process can be applied.

Because of their material and design restrictions, most flexible endoscopes are not temperature-resistant. Therefore, steam sterilization processes at elevated temperatures cannot be applied for sterilization of flexible endoscopes. The following alternative low temperature processes are available:
- Ethylene oxide gas sterilization;
- Hydrogen peroxide gas sterilization with and without plasma;
- Low temperature steam and formaldehyde sterilization.

It must be recognized that low temperature sterilization processes are only effective if thorough cleaning has already been done. Manual reprocessing and use of an EWD before sending the endoscope to a CSSD for sterilization will be important in order to protect reprocessing staff.

Most European countries do not accept immersion of endoscopes into liquid chemical sterilants, because the devices are not wrapped in sterile packages until the next use. A critical point is also the quality of the final rinse water which might impair he sterilization effect.

At present the hydrogen peroxide gas sterilization used on some GI endoscopes has technical limitations. Gastroscopes, colonoscopies, and duodenoscopes have from three to seven long separate channels and therefore exceed the lumen capacity of existing sterilizers.
Further development and research will be needed.

6.11 Transport of Ready-To-Use Reprocessed Endoscopes

RECOMMENDATION

Hand disinfection should be done before reprocessed endoscopes are handled.

RECOMMENDATION

Reprocessed endoscopes should be transported in a disinfected closed container, clearly marked as “clean equipment ready for use.”
Endoscope components should also be transported in this closed container.

Transport in closed containers reduces the risk of recontamination and prevents any damage to the endoscope during the transportation phase.

Hand hygiene compliance in endoscopy is a crucial point. Reprocessed endoscopes can be recontaminated if hand hygiene is insufficient.

If several endoscopes are used during one procedure, each endoscope should be transported in a separate container to avoid any damage.

7. Documentation and Traceability

7.1 Documentation

RECOMMENDATION

The complete reprocessing cycle should be documented:

- Each reprocessing step (including bedside cleaning, manual cleaning, and automated reprocessing in an EWD or ADD) should be recorded manually or electronically, including the names of the persons undertaking each step.
- The process parameters of the EWD and storage cabinets should be documented by printouts or electronically.
- All endoscopes should have a record of their reprocessing showing that they are ready for use on patients.
- The reprocessing record should be documented in the patient's files.

The documentation of the reprocessing procedure should include:

- The patient on whom the endoscope was last used;
- The endoscope identification;
- The whole reprocessing cycle including all manual cleaning steps, and identification of the EWD/ADD and storage cabinet used (if applicable);
- The time-frame for reprocessing and storage.
- Identification of the staff member involved in reprocessing of that endoscope;
- Identification of the staff who check the correct performance of the reprocessing cycle and release the endoscope for use on the next patient.

Quality assurance entails that the evidence of correct reprocessing is included in the file of the next patient. Therefore an interface between electronic documentation of medical endoscopy reports and reprocessing is essential to allow data transfer. In cases of suspicious infection this data exchange is a necessary tool for investigating nosocomial infections.

7.2 Maintenance

RECOMMENDATION

Regular maintenance of all technical equipment, including endoscopes, EWDs, and storage cabinets, should be defined according to the manufacturer's IFU.

RECOMMENDATION

It is the responsibility of the clinical service provider to contact the relevant manufacturer as well as regulatory bodies if:

- The manufacturer's recommendations are unclear;
- Any problems arise while using or reprocessing their equipment;
- Suspicious infections occur in conjunction with a specific device (e. g. endoscope, EWD, ADD, storage cabinet, sterilization device).

In the case of technical problems, endoscopes, EWD, ADDs, storage cabinets, or sterilization devices may pose a potential infection risk. Therefore they must:

- Be cleaned/disinfected and maintained according to manufacturer's IFU on a daily basis;
- Have regular engineering maintenance;
- Undergo regular microbiological surveillance according to EN ISO 15883, and for storage cabinets according to EN 16442.

7.3. Loan Endoscopes and Prototypes

RECOMMENDATION

Prior to first use on patients, loan endoscopes and prototypes should be reprocessed, following the whole reprocessing cycle including manual brushing, and should be checked for correct functioning.

RECOMMENDATION

If a loan endoscope and prototypes differ from endoscopes usually used in the department, endoscopy and reprocessing staff should receive instructions from the supplier about the equipment, including the channel configuration and reprocessing information.

RECOMMENDATION

The clinical service provider must check whether this type of endoscope can be reprocessed in the local EWD.

RECOMMENDATION

The specifications of each loan endoscope and prototypes should be included in the database of the endoscopy department as well as in the database for the local EWDs and storage cabinets (if applicable) in order to enable appropriate documentation.

Staff must be familiar with the channel configuration of loan endoscopes and prototypes in order to ensure safe reprocessing. Because of legal issues, it is necessary to document the use of loan endoscopes and prototypes in all relevant patient and hygiene-focused documentation systems.

The clinical service provider must check whether the type of the loan endoscopes and prototypes can be reprocessed in the local EWD. If necessary, the clinical service provider should contact the EWD/ADD supplier to receive information about compatibility with the process chemicals, and about necessary connectors for the EWD, ADD, and storage cabinets in order to ensure safe reprocessing.

8. Outbreak Management

RECOMMENDATION

The clinical service provider should establish procedures detailing the management of any suspicious infection as well as suspected or identified breaches in reprocessing. The procedure should indicate the management of the potentially affected patients, staff, and equipment.

RECOMMENDATION

If any contamination is found, it is the responsibility of the clinical service provider to take the suspected piece of equipment out of service (e. g. endoscopes, EWD, ADD, storage cabinet,

accessories, etc), until corrective actions have been taken and satisfactory results have been achieved.

RECOMMENDATION

Outbreaks should be managed within the multidisciplinary team of endoscopy departments, hospital hygiene experts, microbiologists, manufacturers, and regulatory bodies, if applicable.

Staff training, adherence to guidelines and manufacturers' IFUs, regular quality assessment with audits, regular microbiological surveillance, and validation of reprocessing cycles are important tools in the prevention of infections. European and national guidelines already provide helpful flowcharts concerning outbreak management.

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Natteri Veeraraghavan

Natteri Veeraraghavan (1913-2004) was an Indian physician, microbiologist and medical researcher, known for his contributions to the understanding of diseases like rabies, tuberculosis and leprosy. He was a former director of the Pasteur Institute of India, Coonoor and the chairman of the World Health Organization International Reference Center on Rabies. He was honoured by the Government of India in 1967, with the award of Padma Shri, the fourth highest Indian civilian award for his contributions to the society.

Natteri Veeraraghavan was born in Parangipettai, in Chennai district in the south Indian state of Tamil Nadu on 1 November 1913 to Natteri Venkatesa Iyer and Balambal. His graduated in medicine (MBBChir) from Andhra University in 1936 and did his senior internship at the Government Mental Hospital, Chennai in 1937. His career started as a research officer at the Pasteur Institute of India at Coonoor in 1937 and became the assistant director of the institute in 1941. While working in that position, he secured a doctoral degree (DSc) in microbiology from Andhra University in 1944.

In 1947, Veeraraghavan was made the director of the institute, a post he held till his retirement in 1972. Post retirement, he served as the director of the Vector Control Research Center, Pondicherry, from 1975 to 1977 and as the director of the Voluntary Health Services Medical Centre from 1977. He was a member of the Indian Council of Medical Research from 1945 to 1981 and sat on the Expert Panel of the World Health Organization on Rabies in 1953, 1956,1959, 1965 and 1972. He was the vice chairman of the panel in 1953 and the chairman in 1959. He also served as a member of the Indian chapter of the World Health Organization Influenza Center from 1959 to 1972 and the Armed Forces Research Committee from 1967 to 1972.

One of the founding members of the Indian Academy of Medical Sciences (present day National Academy of Medical Sciences), Veeraraghavan was known to have involved in extensive research in microbiology. He held many patents for his research and his research findings have been documented by way of several publications. Some of his notable publications are:

- Studies on Leprosy
- Studies on Leprosy: Supplement 3
- In Vitro Cultivation of M. Leprae: An Improved Medium
- Fluorescent antibody staining of rabies virus antigens using lissamine rhodamine B200 as fluorochrome
- The value of 5% semple vaccine in human treatment : comparative mortality among the treated and untreated
- A rapid method for cultivation of M. tuberculosis : an improved medium

Veeraraghavan, a recipient of the Societe Anonyme Poonawalla Memorial Award, was honoured by the Government of India with the civilian award of Padma Shri in 1967. He died at the age of 90 on 6 August 2004, survived by his wife, Kamala, daughter Shantha, and sons, Dr. N.V. Chandran and Dr. Mani Veeraraghavan.

Jokes

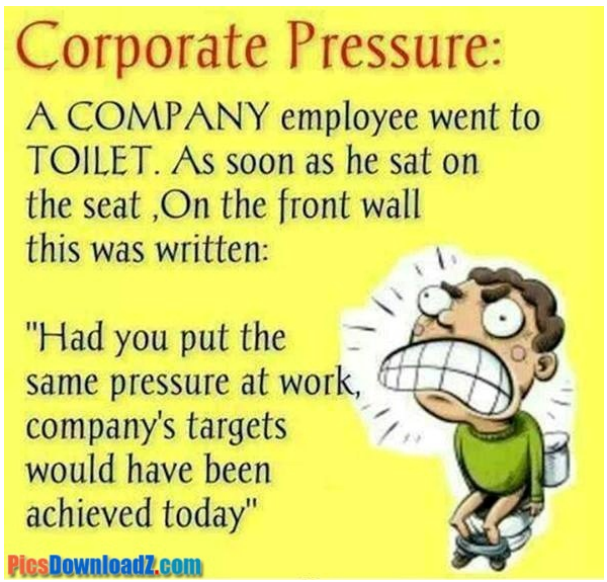
An old grandma brings a bus driver a bag of peanuts every day.
First the bus driver enjoyed the peanuts but after a week of eating them he asked: "Please granny, don't bring me peanuts anymore. Have them yourself."
The granny answers: "You know, I don't have teeth anymore. I just prefer to suck the chocolate around them."

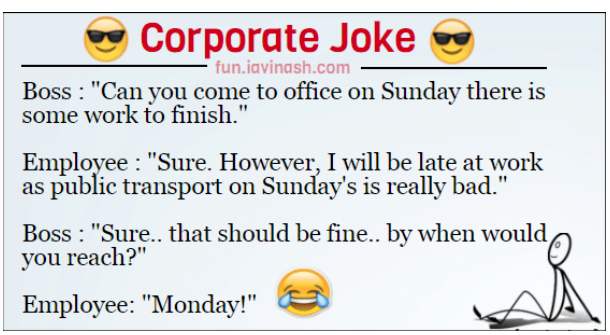
A doctor accidentally prescribes his patient a laxative instead of a coughing syrup.
Three days later the patient comes for a check-up and the doctor asks: "Well? Are you still coughing?"
The patient replies: "No. I'm afraid to."

A man is reading his newspaper and says to his wife: "Michelle, look. Here is an article about how women use about twice as many words per day as men do."
The wife responds: "That's because we have to tell you everything twice"

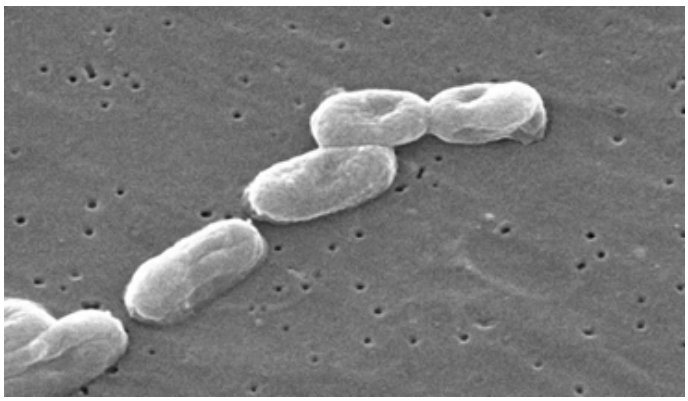
What is the worst combination of illnesses?
Alzheimer's and diarrhea. You're running, but can't remember where.

In a shoe shop:
These shoes might be tight for the next two weeks.
-
Don't worry. I'll start wearing them on the third week





Burkholderia cepacia complex



Burkholderia cepacia* complex (BCC)**, or simply ***Burkholderia cepacia, is a group of catalase-producing, lactose-nonfermenting, Gram-negative bacteria composed of at least 20 different species, including *B. cepacia, B. multivorans, B. cenocepacia, B. vietnamiensis, B. stabilis, B. ambifaria, B. dolosa, B. anthina, B. pyrrocinia* and *B. ubonensis*. *B. cepacia* is an opportunistic human pathogen that most often causes pneumonia in immunocompromised individuals with underlying lung disease (such as cystic fibrosis or chronic granulomatous disease). Patients with sickle-cell anemia are also at risk. The species also attacks young onion and tobacco plants, as well as displaying a remarkable ability to digest oil.

B. cepacia was discovered by Walter Burkholder in 1949 as the cause of onion skin rot, and first described as a human pathogen in the 1950s. It was first isolated in patients with cystic fibrosis (CF) in 1977 when it was known as *Pseudomonas cepacia*. In the 1980s, outbreaks of *B. cepacia* in individuals with CF were associated with a 35% death rate. *B. cepacia* has a large genome, containing twice the amount of genetic material as *E. coli*.

Pathogenesis

BCC organisms are typically found in water and soil and can survive for prolonged periods in moist environments. They show a relatively poor virulence. Virulence factors include adherence to plastic surfaces (including those of medical devices) and production of several enzymes such as elastase and gelatinase. Also relevant might be the ability to survive attacks from neutrophils.

Person-to-person spread has been documented; as a result, many hospitals, clinics, and camps have enacted strict isolation precautions for those infected with BCC. Infected individuals are often treated in a separate area from uninfected patients to limit spread, since BCC infection can lead to a rapid decline in lung function and result in death.

Diagnosis

Diagnosis of BCC involves culturing the bacteria from clinical specimens, such as sputum or blood. BCC organisms are naturally resistant to many common antibiotics, including aminoglycosides and polymyxin B. and this fact is exploited in the identification of the organism. The organism is usually cultured in *Burkholderia cepacia* agar (BC agar) which contains crystal violet and bile salts to inhibit the growth of Gram-positive cocci and ticarcillin and polymyxin B to inhibit the growth of other Gram-negative bacilli. It also contains phenol red pH indicator which turns pink when it reacts with alkaline byproducts generated by the bacteria when it grows.

Alternatively, oxidation-fermentation polymyxin-bacitracin-lactose (OFPBL) agar can be used. OFPBL contains polymyxin (which kills most Gram-negative bacteria, including *Pseudomonas aeruginosa*) and bacitracin (which kills most Gram-positive bacteria and *Neisseria* species). It also contains lactose, and organisms such as BCC that do not ferment lactose turn the pH indicator yellow, which helps to distinguish it from other organisms that may grow on OFPBL agar, such as *Candida* species, *Pseudomonas fluorescens*, and *Stenotrophomonas* species.

Treatment

Treatment typically includes multiple antibiotics and may include ceftazidime, doxycycline, piperacillin, meropenem, chloramphenicol, and trimethoprim/sulfamethoxazole (co-trimoxazole). Although co-trimoxazole has been generally considered the drug of choice for *B. cepacia* infections, ceftazidime, doxycycline, piperacillin, and meropenem are considered to be viable alternative options in cases where co-trimoxazole cannot be administered because of hypersensitivity reactions, intolerance, or resistance. In April 2007, researchers from the University of Western Ontario School of Medicine, working with a group from Edinburgh, announced that they had discovered a potential method to kill the organism, involving disruption in the biosynthesis of an essential cell membrane sugar.

Babies born by C-section have more potentially infectious bacteria in their guts

Babies born by Caesarean section may miss out on many of mom's helpful gut microbes. Instead, these infants' guts harbor more bacteria that commonly lurk in hospital rooms, scientists found.

The finding, described September 18 in *Nature*, adds weight to the idea that C-sections, and the antibiotics that often come with the procedure, 'may change the type of bacteria that first take up residence in a newborns gut. This collection of microbes helps form the microbiome, and details of this early colonization might be important for long-term health, some scientists suspect.

Still, the results shouldn't dissuade women from receiving C-sections if needed. “Caesarean sections are a life-saving and medically necessary intervention,” says Lisa Stinson, a molecular microbiologist and reproductive biologist of the University of Western Australia in Perth. But “we need a better understanding of their long-term effects on infants.”

Microbiome imbalances have been linked to disorders such as asthma, allergies and other inflammatory diseases *(SN: 2/17/17)*. But scientists don't know whether a baby's nascent microbiome could ultimately influence these disorders. Nor is it clear whether birth details change that early microbial colonization.

To get a snapshot of these early bacterial moments, genomicist Yan Shao of the Wellcome Sanger Institute in Cambridge, England, and colleagues studied the gut bacteria living in 596 full-term, healthy babies born in U.K. hospitals. In the 314 babies born vaginally, helpful gut microbes such as *Bifidobacterium* and *Bacteroides* made up 68 percent on average of the total gut bacteria. These bacteria were scarcer in the guts of babies born by C-section. Instead, species commonly found in hospital settings, including potentially harmful *Enterococcus* and *Clostridium*, accounted for an average of 68 percent of the total gut bacteria.

Surprisingly, neither group of babies had much bacteria from their mothers' vaginas, an absence that calls into question the usefulness of delivering vaginal microbes to newborns *(SN: 3/30/16)*. Smearing vaginal fluids onto babies born via C-section has been studied as a way to restore normal gut microbiota. But the new study “found no evidence to support controversial 'vaginal swabbing' practices,” Shao says.

Along with the birth mode itself, antibiotics delivered during birth also shape which bacteria set up shop in an infant's gut, the study suggests. In many places, mothers who undergo C-sections receive antibiotics to prevent infections. Along with removing potential threats, these drugs can kill helpful bacteria. In the study, babies born vaginally but whose mothers also took antibiotics had fewer helpful *Bacteroides* bacteria. That suggests that some of the bacterial differences are “related to maternal antibiotic exposure — not lack of exposure to vaginal bacteria at birth,” Stinson says.

Antibiotics could also affect the microbial mixtures in mothers' breast milk, Stinson says, which could then influence babies' gut microbiomes. In the study, breastfeeding had a small effect on babies' gut bacteria.

As the babies grew older and began eating solid food, differences in gut bacteria composition shrank, researchers found by looking at a smaller set of the babies.

During the study, all of the babies were healthy. It's not known whether the bacteria that have the potential to cause infections in these babies would actually cause trouble later in life. Even so, starting out life with the wrong repertoire of microbes likely has consequences, says microbiologist Maria Dominguez-Bello of Rutgers University in New Brunswick, N.J.

Best Practices in Wound Care Management (Issue III)

TISSUE MANAGEMENT

Accurately assessing the wound bed will help differentiate between viable (eg granulation and epithelial) tissue and nonviable tissue (eg black eschar/necrosis and slough). Non-viable or devitalised tissue provides an opportunity for anaerobic and aerobic bacteria to grow, which can delay wound healing and result in significant malodour.

INFECTION/INFLAMMATION

There is no hard-scientific test to diagnose infection, so clinical judgement is needed to interpret signs and symptoms. The list of signs and symptoms is itself a topic of debate, so the challenge is to make the best use of the clinical information available at the assessment, create a plan, and reassess regularly to determine treatment response and alter the care plan accordingly.

Several classic signs and symptoms are easily identifiable as wound infection, but not all wounds will exhibit all these signs at any one time. Localised infection is often characterised by the classical signs and symptoms of inflammation, pain, heat, swelling, redness and loss of function; these indicators are more likely to be apparent in acute wound infection than in chronic wound infection.

Additional, possibly more sensitive, criteria have been suggested for identifying wound infection, including abscess formation, cellulitis, discharge, delayed healing, discolouration, friable granulation tissue that bleeds easily, unexpected pain, pain that has changed in nature, tenderness, pocketing at the base of the wound, bridging of epithelium or soft tissue, abnormal smell and wound breakdown (Cutting et al, 2005). These kinds of so-called secondary wound infection characteristics might be better indicators in chronic wounds, particularly when classic signs are absent (Gardner et al, 2001).

There is little consensus to define whether wound microbiology is of use in guiding clinical decisions (Moore et al, 2007) because swabbing a wound will identify some or all bacteria within the wound but might not always indicate the clinically significant species (Wounds UK, 2010). The value of a surface swab is debatable; tissue biopsy for quantitative microbiology is considered the most appropriate sampling method to identify wound infection and causative organism (Bowler et al, 2001; cited in Moore et al, 2007). This should be done after wound debridement ideally, from the tissue or bone from the base of the wound, or a deep wound swab and before systemic antibiotics are initiated (Saad et al, 2013).

MOISTURE IMBALANCE

High levels of exudate are associated with bacterial colonisation of a wound (Cutting and White, 2002). When a wound becomes infected, exudate will increase rapidly, particularly in those with underlying comorbidities such as diabetes. Discolouration and highly viscose exudate often indicate infection, especially when the exudate changes from pale amber colour to, for example, green (indicative of *P. aeruginosa*). However, a wound can be infected even if thick or discoloured exudate is absent (Wounds UK, 2013b). Further, diagnosis must also rule out conditions, eg lymphoedema or chronic venous insufficiency, that can cause excess exudate. Clinicians must effectively manage exudate to create the optimal moist environment necessary for wound healing and to protect the surrounding skin from the risks of maceration and excoriation. Achieving these goals requires a detailed knowledge of dressing materials and their performance (Wounds UK, 2013b).

EDGE OF WOUND

Lack of improvement in wound dimensions and non-progression of the wound edge indicate failure to heal. The presence of devitalised tissue, such as areas of necrosis or slough, can delay wound healing. Healing rates are a reliable early predictor of complete wound closure; wound margin advance, initial healing rate, percent wound surface reduction and wound healing trajectories are powerful predictors of healing at 12 weeks (Cardinal et al, 2007).

BEST PRACTICE STATEMENT APPLICATION TO PRACTICE: ASSESSING THE PATIENT AND WOUND

Best practice statement	Reason for best practice statement	How to demonstrate best practice
Holistically assess each patient and rule out the need to treat underlying conditions before prescribing any wound products or medications	To prevent inappropriate products or medications being used	Clearly document the assessment process, including a plan of care, review dates for future assessments and the rationale for dressing choice Regularly review medications
Clinicians must ensure they understand the wound-healing process and are competent in accurate assessment	To ensure factors that might impede the complex wound-healing process are identified and, where possible, addressed	Clearly document the assessment process, including the wound bed condition, using an assessment tool (eg TIME) Refer the patient, in a timely manner, to the appropriate member of the multidisciplinary team if there is delayed wound healing or signs of infection

BIOFILMS AND WOUND INFECTION

Key Points:

1. Biofilms have been implicated in infections of many tissues; they are very likely to be implicated in chronic wounds.
2. Biofilms cannot be visualized or detected in the wound.
3. Treatment should anticipate biofilm's presence. The coordinated use of debridement and specific topical antimicrobials is advocated.

Biofilms are complex polymicrobial communities that develop on or near wound surfaces. Biofilms may not present with clinical

signs of infection (Phillips et al, 2010), but their presence has been implicated in chronicity (Bjarnsholt et al, 2006; James et al, 2008). They are invisible to the naked eye, cannot be detected by routine cultures and are extremely difficult to eradicate (Phillips et al, 2010). Not all biofilms are harmful, but some communities can be tantamount to wound infection, delaying healing as a result (Wolcott et al, 2008). The host's attempt to rid the wound of a biofilm stimulates a chronic inflammatory response, which releases high levels of reactive oxygen species (ROS) and proteases (MMPs and elastase). Although these substances help break down the attachments between the tissue and the biofilms, the ROS and proteases also damage normal and healing extracellular matrix tissues, potentially delaying ealing (Wolcott et al, 2008).

DEVELOPMENT OF BIOFILMS

The extracellular polymeric substance that contributes to the structure of the biofilm lets microbial species exist in close proximity to one another. This matrix which can be largely impermeable to antibiotics acts as a thick, slimy protective barrier and attaches the biofilm firmly to a living or non-living surface. Biofilms are dynamic and heterogeneous communities. They form quickly within two to four hours and evolve into a fully mature biofilm community within two to four days (Wolcott et al, 2008). They rapidly recover from mechanical disruption and reform mature biofilm within 24 hours. Communities can consist of a single bacterial or fungal species or, more commonly, can be polymicrobial (Dowd et al, 2008).

PREVALENCE OF BIOFILMS

Using electron microscopy and confocal scanning laser microscopy, biofilms have been found in 60% of biopsy specimens from chronic wounds, compared with only 6% of biopsies from acute wounds (James et al, 2008). Because biofilms are thought to significantly contribute to multiple inflammatory diseases, it is likely that almost all chronic wounds have biofilm communities on at least part of the wound bed (Phillips et al, 2010). Although biofilms might be an important contributor to wound chronicity, not all wounds with delayed healing can be assumed to contain biofilm. Further, the distribution of biofilms when they do exist in wounds (49% of wounds in James et al [2008] were without biofilms) seems to depend on the species, with *P. aeruginosa* found in deeper wound areas than *S. aureus* (Fazli et al, 2009). In addition, it is not known whether the presence of a biofilm in a wound will always lead to problems.

WHEN TO SUSPECT A BIOFILM

Chronic skin wounds often lack overt clinical signs of infection and might have low bacterial burdens as measured by standard clinical microbiology laboratory assays (WUWHS, 2008). The term 'biofilm' was developed in an attempt to acknowledge that bacteria play a critical role in the failure to heal of wounds that do not have obvious signs of infection.

MANAGING BIOFILMS

Evidence to date suggests that debridement or vigorous physical cleansing, are the best methods for reducing biofilm burden (Wolcott et al, 2009). Before commencing debridement, however, the patient should be assessed to determine the wound's healing potential. Wound irrigation using sterile saline or tap water can be used to clean chronic wounds to allow assessment and debridement. It is important to remember to not use gauze or cotton wool during cleaning, to avoid leaving debris in the wound bed, which might in turn cause infection. Topical antiseptic agents are considered unnecessary for general wound cleansing, but might be of value when irrigating an infected cavity wound or chronic wounds at risk of infection (Bradbury and Fletcher, 2011).

Active debridement is contraindicated in cases of severe vascular compromise. When indicated, remove non-viable tissue as quickly and efficiently as possible using an appropriate debridement method to assist with assessment, reduce bioburden/biofilm and accelerate healing (Wounds UK, 2013). Clinicians can use autolytic, mechanical, sharp, larval therapy (biosurgical), ultrasonic, hydrosurgical and surgical debridement. Each clinician must be competent, skilled, educated and trained in each technique. The debridement method chosen should be determined by the patient's clinical need and choices, and not limited by the skills of the clinician (Gray et al, 2011).

Debridement with a monofilament fibre pad 'shows the potential to advance mechanical debridement as a viable technique, by providing a rapid, safe and easy-to-use method with limited pain for the patient' according to the EWMA (2013b). However, if this method is not available and the clinician has received no training in specific debridement skills, assistance and advice must be sought from a healthcare professional with expertise in debridement techniques.

There are relatively few wounds that are not safe to debride if the correct method is chosen. As a general rule, if the wound is not covered in granulation tissue, debridement can be performed to progress a wound towards healing (Wounds UK, 2013a).

Keep in mind that no form of debridement or cleansing is likely to remove all biofilm, so remaining bacteria/biofilm could reform into mature biofilm in a matter of days. Topical antimicrobial interventions are potentially more effective at this post-cleansing/post-debridement stage (Wolcott et al, 2009), and should be considered for application to the wound, either as an antiseptic wound cleansing agent with a surfactant component and/or antimicrobial dressing.

Several antimicrobial agents have been shown to inhibit or even prevent biofilms in vitro (EWMA, 2013a). Sustained-release cadexomer iodine has been shown to be more effective than silver (Hill et al, 2010) or PHMB in disrupting mixed biofilms (Phillips et al, 2010); silver absorbent dressings have been shown to prevent biofilm formation by all singled and mixed biofilm cultures (Driffield et al, 2007). However, PHMB has also been shown to have microbiocidal activity on chronic wounds and burns, and to reduce biofilm in wounds exhibiting chronicity (Lenselink and Andriessen, 2011). Inert absorbent dressings have also been shown to exhibit both antifungal and antimicrobial effects, inhibiting *P. aeruginosa*, *K. pneumoniae* and *E. coli* presence in wounds, and significantly reducing *S. aureus* and *C. albicans* (Wiegand et al, 2012).

Use of topical antimicrobial agents in the presence of biofilms should occur only after biofilm disruption. These key steps summarise the management of biofilms in practice (Dowsett, 2013)

- Seek to prevent biofilm development whenever possible.
- Prepare the wound bed, considering the use of cleansing, debridement and topical antimicrobials where appropriate.
- Vigorously clean the wound with products designed to disrupt biofilm.
- Select debridement method based on wound type, best practice and patient preference.
- After debridement, consider topical antimicrobial treatment, as the biofilm is more vulnerable at this stage and can be

managed with topical antimicrobial application more effectively than it could have been pre-debridement.

SELECTION AND USE OF TOPICAL ANTIMICROBIALS

Key points:

1. Topical antimicrobials present limited potential for systemic absorption and toxicity.
2. Topical antimicrobials are ideal for providing high and sustained concentration of antimicrobial at the site of the infection, potentially limiting the amount of overall antimicrobial needed in combination with systemic treatment.
3. Topical antimicrobials should be used only when signs and symptoms suggest that wound bioburden is interfering with healing, or when there is an increased risk of serious outcomes.
4. Not all wounds exhibit all symptoms of critical colonisation or infection, and there is not necessarily a standard progression of indicator severity.
5. Clinical colonisation must be determined in the context of all information about the wound and patient.

BEST PRACTICE STATEMENT APPLICATION TO PRACTICE: BIOFILMS AND WOUND INFECTION

Best practice statement	Reason for best practice statement	How to demonstrate best practice
Prevent biofilm development wherever possible	Biofilms can delay healing	Clearly track and document wound progress towards healing
Treatment should aim to disrupt biofilm burden through regular, repeated debridement and/or cleansing	To reduce the presence of biofilm and help prevent the reformation and attachment of biofilm	Patient documentation should reflect clinical rationale for treatment choice
Select debridement or cleansing method based on wound type, the clinician's knowledge and patient preference	To encourage effectiveness of treatment and patient concordance with the treatment chosen	Patient documentation should reflect clinical rationale for treatment choice as well as record of discussion with the patient
Consider topical antimicrobial treatment after cleansing or debridement	To better manage biofilm burden, as it is more vulnerable at this stage	Clearly document the rationale for pursuing treatment with a topical antimicrobial

6. Topical antimicrobials vary according to the concentration and availability of the active ingredients, mode and duration of action, and ability to handle exudate, odour or pain, and should be selected specific to the needs of each wound and patient, weighing the advantages and drawbacks of use.
7. To avoid serious consequences of infection, clinicians must also identify high-risk patients for whom
8. systemic antibiotics might be indicated.
9. Using topical antimicrobials does not guarantee a healing outcome, but is currently a reasonable, practical method for reducing the risks posed by infection at specific times on the wound care pathway.

In clinical practice, attributing either positive (clinical improvement) or negative (treatment failure) outcomes to topical antimicrobial treatment is currently not possible; it is a matter of reasoned opinion based on good clinical assessment. The important elements or treatment goals of using topical antimicrobials in a management plan are the potential to:

- Prevent progression from localised colonisation to more invasive infection states, thereby reducing the antibiotic usage
- Return to normal healing progression
- Treat critical colonisation/local infection without resorting to antibiotics
- Achieve faster resolution of local infection in conjunction with antibiotics (the literature does not prove this outcome advantage, but it is logical to expect more rapid resolution when reducing the wound-base pathogen reservoir and minimising antibiotic-resistant strains in the wound bed)
- Improve the patient experience by correctly diagnosing the cause of and controlling dour, exudate leakage and pain.

Clinically determining the patient's ability to resist bacterial invasion is the most important contributing factor in determining microbial balance (White, 2013). As such, topical antimicrobials should not be used 'just in case' in a wound that is healing as expected, unless clinically justified due to a patient's high risk (Butcher and White, 2013). For critically colonised or locally infected wounds, topical antimicrobials can be used, as part of a treatment plan as determined using the TIME framework, to help control microbial load (eg, biofilm) and protect the wound from further damage or contamination.

WHAT ARE ANTIMICROBIALS?

Antimicrobials are agents capable of killing (biocidal) or inhibiting (biostatic) microorganisms. They have broad-spectrum activity against potentially infection-causing Gram-positive, Gram-negative, aerobic and anaerobic, planktonic and sessile (Wolcott et al, 2008) bacteria, and fungi and spores commonly found in the wound bioburden. As many antimicrobials can adversely affect human tissue, a compromise between antibacterial efficacy and cytotoxicity might have to be accepted (Müller and Kramer, 2008). The umbrella term includes:

- Disinfectants, substances used to inhibit or kill microbes on inanimate objects (eg dressing trolleys and instruments)
- Antiseptics, agents used to inhibit or kill microorganisms within a wound (biofilm) or on intact skin (eg, iodine)
- Antibiotics, naturally occurring (produced by

microorganisms) or synthetically produced substances that can act selectively and can be applied topically (not normally recommended in wound care) or systemically. Microbial resistance is common (Vowden et al, 2011).

According to Lipsky and Hoey (2009), topical antimicrobials are ideal for providing high and sustained concentration of antimicrobial at the site of infection, potentially limiting the amount of overall antimicrobial needed in combination with systemic treatment perhaps eliminating systemic therapy altogether. Further, topical antimicrobials present limited potential for systemic absorption and toxicity (Lipsky and Hoey, 2009). Other benefits include:

- Relatively easy use
- Wide availability
- Generally lower cost than antibiotics
- Less risk for developing resistance (Vowden et al, 2011).

However, because of their surface nature, antimicrobials cannot be used to treat deep-tissue infection and might cause local hypersensitivity or contact dermatitis reactions at the skin and wound bed or alter normal skin flora, interfering with wound healing (Lipsky and Hoey, 2009).

Wound bioburden can also be managed via passive mechanisms without necessarily inhibiting the wound's microbial flora. Modes of action include bacterial sequestration (eg via mechanically modified cellulose fibres and selected gelling agents) within the dressing or binding of wound pathogens to a dressing substrate (eg via dialkylcarbamoylchloride, known as DACC). Bacteria and fungi that are bound in the latter manner are rendered inert on the wound contact layer, so no further replication takes place, and are removed from the wound environment when the dressing is changed.

WHEN ARE TOPICAL ANTIMICROBIALS INDICATED?

There are two broad categories of wounds in which topical antimicrobials should be considered for use. In the first kind of situation, no obvious underlying patient historical or lifestyle factors would compromise wound healing. In the second, underlying comorbidities and patient historical and lifestyle factors are present that might inhibit wound healing.

Situation 1

- Topical antimicrobials should be used only when signs and symptoms suggest that wound bioburden is interfering with healing:
- Cessation of progress, where previously response to that same therapy was evident and when other potential reasons have been explored and eliminated
- Failure to heal despite proper treatment — meaning wound care has included adequate debridement, removal of foreign bodies, pressure offloading (not leg ulcers), appropriate dressings, and treatment of any arterial or venous insufficiency or metabolic derangements (Lipsky and Hoey, 2009)
- Signs and symptoms of critical colonisation or localised infection (covert infection)
- Signs and symptoms of overt local or spreading infection.

CONSIDERATIONS WHEN SELECTING TOPICAL ANTIMICROBIALS

Products vary according to the concentration and availability of the active ingredients, mode and duration of action, and ability to handle exudate, odour or pain, and should be selected specific to the needs of each wound, weighing the advantages and drawbacks of use. To avoid serious consequences of infection, clinicians must also identify high-risk patients, such as those with poor vascularity or compromised immune systems, for whom the systemic antibiotic use might be indicated. For spreading infection, systemic antibiotics are normally selected empirically (EWMA, 2013a).

SUMMARY AND CONCLUSIONS

Promptly diagnosing and managing infection is vital to avoid complications. Clinicians must be knowledgeable of the signs and symptoms of infection, and those patients in whom these might be subtle or absent. It is imperative that clinicians be aware of the impact of comorbidities, medication and therapies on wound healing and infection. Most wounds are colonised by bacteria (ie contain biofilm) and, yet, the majority are not infected, and healing progresses normally (Angel et al, 2011).

Understanding the correct use of antimicrobial therapy is crucial not only in preventing wound infection but also in promoting wound healing for the patient. All wounds are colonised. Critical colonisation/local infection may can delay healing, cause complications and significantly affect daily living for patients, with increased pain and anxiety, exudate with the potential for leakage and odour. Preventing and managing critical colonisation/local infection is closely linked to quality of care and patient safety (EWMA, 2013a).

Guide to topical antimicrobials

This table presents the key points of widely used types of topical antimicrobials, listed alphabetically. Always check manufacturer instructions for use and contraindications. Select the antimicrobial product on an individual basis, customising the agent and dressing choice according to patient, wound and environment needs.

Active control	Mode of delivery	Rationale for use	Wound types	Guidance for use	Contraindications
Enzyme alginogel	Alginate gel	▪ Autolytic debridement ▪ Maintain moisture balance ▪ Reduce microbial burden ▪ Protect wound edges and epithelial cells	▪ Pressure ulcers ▪ Diabetic ulcers ▪ Traumatic wounds ▪ Arterial ulcer ▪ Second-degree burns ▪ Radiotherapy and oncology wounds ▪ Treat pregnant patients, as there is no absorption into the body	▪ Apply to wound and cover with a secondary dressing ▪ Check frequently to ensure correct level of gel ▪ Can be used long-term due to no body absorption	▪ Patients with known sensitivity alginate dressing or polyethylene glycol ▪ Wounds on the eyelid or where there is danger of contact with the eye

Active control	Mode of delivery	Rationale for use	Wound types	Guidance for use	Contraindications
Iodine - Povidone iodine - Cadexomer iodine	Solution, cream, ointment, spray or impregnated dressings	▪ Treat localised infection, or spreading infection when healing is delayed ▪ Prevent wound infection or recurrence in susceptible patients ▪ Rapidly kill microorganisms, including MRSA ▪ Prevent bacterial resistance ▪ Suppress biofilm formation	▪ Venous leg ulcers ▪ Diabetic ulcers ▪ Cavity wounds (cadexomer only)	▪ Use initially for one week only, with dressing changes 2 to 3 times weekly ▪ If the wound does not improve after 10 to 14 days, re-evaluate the wound and change the dressing regimen/ systemic treatment	▪ Long-term use (due to perceived issues with toxicity, systemic absorption and delayed healing) ▪ Known or suspected iodine sensitivity ▪ Children ▪ Before after radio-iodine diagnostic tests ▪ Patients with significant renal disease ▪ Patients with thyroid Disease
Polyhexamethylene biguanide (PHMB)	Solution, gel or impregnated dressings	▪ Cleanse/ decontaminate the wound ▪ Suppress biofilm formation ▪ Reduce wound odour ▪ Removal of encrusted dressings (solution only) ▪ Manage wound bioburden ▪ Provide an antimicrobial barrier	▪ Partial-thickness burns ▪ Post-surgical wounds ▪ Traumatic wounds ▪ Skin donor/recipient sites ▪ Leg ulcers ▪ Pressure ulcers ▪ Diabetic foot ulcers ▪ Scleroderma wounds ▪ Paediatric wounds	▪ Apply solution to wound and leave for 10 to 15 minutes (can be warmed to body temperature) ▪ Gel can be applied to deep or tunnelling wounds and cavity wounds. Leave in place and apply secondary dressing ▪ Dressings can be left in place for up to 5 to 7 days	▪ Patients with known PHMB sensitivity ▪ Combined with other wound cleansers (eg Dakin's) or ointments ▪ With caution/under medical supervision in pregnant and lactating women or babies ▪ With peritoneal or joint lavage

Going forward, preventing MRSA bacteraemia through control of MRSA in wounds is also on the government agenda in England. MRSA bacteraemia is being treated essentially as a 'never event' because the policy is zero tolerance — if an orangisation goes over its limit as set by the Department of Health (commonly, the figure is zero cases), a fine will be levied by the commissioners of care (NHS England, 2013). The CCG is also penalised for cases in its commissioning area, as 12.5% of quality premiums will not be paid to the CCG. The full premium can be earned only if no cases of MRSA bacteraemia are assigned to the CCG, and if *C. difficile* cases are at or below defined thresholds for the CCG (NHS England, 2013).

Reducing inappropriate use of antibiotics for wound care will contribute to meeting *C. difficile* and MRSA targets (by not creating further resistance issues) and, therefore, reducing fines levied on providers and helping ensure quality premiums are awarded to CCGs. Using topical antimicrobials appropriately can help prevent selecting for resistant bacteria while promoting factors such as reduced bioburden that encourage wound healing.

Appropriate and effective use of topical antimicrobial agents and dressings is important to meeting clinical and patient needs.

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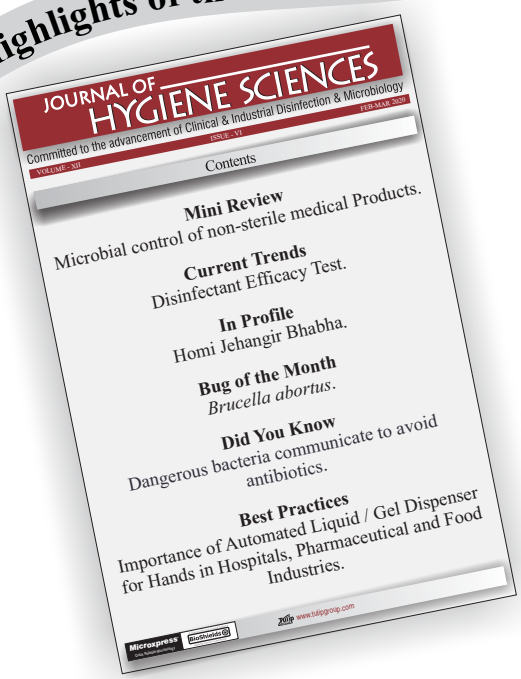
Highlights of the coming issue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