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Editorial

Contents

We would like to thank all our esteem readers for their continuous support & encouragement in making this Journal a successful effort. Here's another interesting issue of JHS coming your way…….

'Mini review' section: Antimicrobial susceptibility testing methods help to define the optimal treatment and identify antimicrobial resistance. Conventional, gold-standard methods and broth microdilution, are still widely applied in clinical settings. Although recently developed methods based on microfluidics and microdroplets have shown advantages over conventional methods in terms of testing speed, safety and the potential to provide a deeper insight into resistance mechanisms, extensive validation is required to translate this research to clinical practice.

Current Trends Section: Automated Endoscope Reprocessors (AERs) are important devices widely used in the health care setting to reprocess endoscopes, such as duodenoscopes, and endoscope accessories, to decontaminate them between uses. AERs are designed to kill microorganisms in or on reusable endoscopes by exposing their outside surfaces and interior channels to chemical solutions. This is a continuation of the earlier issue.....

In Profile: Barbara McClintock (June 16, 1902 – September 2, 1992) was an American scientist and cytogeneticist who was awarded the 1983 Nobel Prize in Physiology or Medicine. McClintock received her PhD in botany from Cornell University in 1927. There she started her career as the leader in the development of maize cytogenetics, the focus of her research for the rest of her life.

Bug of the Month: *Legionella pneumophila* is a thin, aerobic, pleomorphic, flagellated, non-spore-forming, Gram-negative bacterium of the genus Legionella. *L. pneumophila* is the primary human pathogenic bacterium in this group and is the causative agent of Legionnaires' disease, also known as legionellosis.

Did you Know: Elevated estrogen levels in the womb is linked to autism. Elevated latent prenatal steroidogenic activity has been found in the amniotic fluid of autistic boys, based on measuring prenatal androgens and other steroid hormones. Prenatal oestrogens need to be investigated, as they play a key role in synaptogenesis and corticogenesis during prenatal development, in both males and females.

Best Practices: Effectively managing and treating wound infection can challenge clinicians, with myriad products and pharmaceutical interventions available. The results of the Health Protection Agency's Point Prevalence Survey on healthcare-associated infections and antimicrobial use estimated the total number of antimicrobials prescribed as 25,942 for 18,219 patients, with the prevalence of antimicrobial drug and device use being 34.7%. However, indiscriminate use of antimicrobials — in particular, antibiotics— has led to the rising prevalence of resistant organisms. This is a continuation of the earlier issue.....

"There is nothing in the world so irresistibly contagious as laughter and good humor." so ease your mind with some light humour in our **Relax Mood section**.
Looking forward for your feedback & suggestions.

Methods for *In vitro* Evaluation of Antimicrobial Activity (Issue III)

Antimicrobial susceptibility testing methods help to define the optimal treatment and identify antimicrobial resistance. Conventional, gold-standard methods and broth microdilution, are still widely applied in clinical settings. Although recently developed methods based on microfluidics and microdroplets have shown advantages over conventional methods in terms of testing speed, safety and the potential to provide a deeper insight into resistance mechanisms, extensive validation is required to translate this research to clinical practice.

Introduction

Successful management of patients with serious bacterial infections is one of the most challenging aspects of clinical medicine today. In large part it depends upon prompt, accurate clinical and microbiological diagnosis, and upon timely institution of appropriate antibacterial therapy. To utilize antibacterial drugs safely and effectively, the clinician must have adequate knowledge of the characteristics of absorption, distribution, metabolism, and excretion of the various agents, as well as an understanding of their mechanisms of action, relative efficacy, and potential side effects. Above all, he must know whether or not the pathogen in question will be inhibited or killed by concentrations of the antibacterial drug that can be safely achieved at the site of infection. Fortunately, for many pathogens the degree of susceptibility to antibacterial drugs can be determined *in vitro*.

First introduced in 1929, *in vitro* antimicrobial susceptibility testing (AST) methods are still considered to be the most valuable in determining the efficacy of antibiotics or antimicrobial compounds against various microorganisms. In general, *in vitro* AST methods combine one or more antimicrobial agents or materials with bacteria to assess bacterial growth. The efficacy of an agent to kill the bacteria over time can be measured in various ways.

Microcalorimetry

Microcalorimetry utilizes the thermal events of bacterial growth to detect bacterial susceptibility. The test pioneered as an alternative AST method in the mid-1970s. Several studies have been conducted in the past decade using a microcalorimetric assay to determine the MICs of various *S. aureus* and *Escherichia coli* strains, and to detect methicillin-resistant *S. aureus* (MRSA). During the exponential growth phase of bacteria, an exponential increase in heat production takes place due to expanded cellular metabolism. This heat flow can be measured by a calorimeter, and plotting the heat pattern over time allows the generation of a bacterial growth curve. MRSA and methicillin sensitive *S. aureus* (MSSA) were shown to exhibit different heat development profiles after 5 hours and after 4 hours. The possibility to differentiate antibiotic-susceptible and resistant bacterial strains in such a short time is a major advantage and makes it an interesting tool for screening. Microcalorimetry is an informative method, which provides MIC values, the general rate and curve of bacterial growth, antibiotic susceptibility and resistance. Even though microcalorimetric results were found to be in line with standardized methods, there are no official documents of standardization available. Additionally, the amount of research done in measuring heat production for an AST method is limited.

Polymerase chain reaction (PCR)-based techniques

Polymerase chain reaction (PCR)-based techniques for AST are commercially available in the form of assays and automated machines, and are highly favored in clinical settings. PCR, both real-time and conventional, are used to amplify DNA sequences that are specific to a particular pathogen and its susceptibility or resistance to a drug. An example includes the mecA gene that encodes the penicillin-binding protein PBP2a, which reduces the affinity for beta-lactam antibiotics. This gene is commonly found in MRSA and it is highly advantageous in that it can be quickly identified in clinics. Furthermore, the extent and intensity of gene expression and not just its presence are important parameters, as some genes need a high expression in order to produce resistance. One major advantage of PCR-based methods is that they can be conducted and evaluated rapidly. Results can be obtained with high specificity and sensitivity within 2 hours.

Furthermore, samples do not always have to be purified, but can be primary, non-sterile, clinical samples, and, thus, can contain bacterial mixtures. Real-time PCR can detect differences between susceptible and resistant strains with very short incubation time and has been applied for a variety of bacterial types, particularly MRSA and vancomycin-resistant MRSA (VMRSA). It can also distinguish between live and dead bacteria. On the other hand, PCR techniques cannot provide information about mechanisms of resistance. Furthermore, the number of genes and mechanisms involved in resistance can be quite complex depending on the strain, which can lead to misinterpretation of data. Knowing which genes to analyze is a prerequisite to minimize the possibility of detecting false resistance, and this remains one of the major drawbacks of this method. Nevertheless, PCR-based AST methods are a safe, efficient and reliable screening tool in clinical settings

Optical methods

Since their introduction in the mid-1970s, optical methods have developed into sophisticated tools over the years. Generally, light beams are used to detect, stimulate or trap bacterial cells. Common variables measured are bacterial motion, molecular vibrations, bacterial density and fluorescence intensity. Depending on the method, bacterial strains can be identified and a bacterial growth curve as well as quantitative MICs can be determined. Because they rely on expensive and complex spectroscopic readout methods, optical methods are mostly applied in research settings.

Optical density (OD)

Optical density (OD) measurements with a spectrophotometer can determine the growth curve of bacteria over time in a liquid sample. Initial attempts to measure the bacterial growth by OD were performed in 1974, but found greater application after the turn of the century. The OD of a bacterial suspension is determined at regular time points upon exposure to an antimicrobial compound, and can, thus, discriminate resistant and sensitive strains. An OD_{600} of 1 conforms to the exponential growth phase of the bacteria. OD measurements have been done for various *E. coli* and *S. aureus* strains, but also *S. epidermis, S. enterica* and *E. faecalis*. More research is needed to validate the method for a greater variety of bacteria. Although there is no official

standardized protocol for OD measurements, protocols for bacterial suspensions are available from the CLSI, as previously shown for the dilution methods. The main advantage of this method is the fast indication for growth, determining the exponential phase within a couple of hours. It is also non-destructive and inexpensive, which makes it an interesting screening tool. However, the determination of bacterial growth is only an estimation of CFU in solution and cannot determine MICs. The method is not suitable for very low concentrations of bacteria, and it is likely to become unreliable when bacteria are cultured with a dissolving substrate that could interfere. Additionally, coculture and infection studies with human adherent cells that normally proliferate only when attached to surfaces are not possible, as the bacteria need to stay in solution.

Biomimetic polymer sensor

The biomimetic polymer sensor is a fast, automated assay based on the principles of agar plate CFU counting. Membrane-active compounds such as peptides and toxins that are secreted by bacteria interact with agar-embedded nanoparticles, made of phospholipids and chromatic polymer polydiacetylene (PDA). During interaction with these compounds, PDA undergoes a transition from blue to red and starts to emit intense fluorescence at 560 nm and 640 nm, which can be detected by common high-throughput screening instruments, such as multi-well fluorescence plate readers. The initial fluorescence signals indicating the colour change are detectable in less than 6 hours, faster than the bacterial colony formation visible to the naked eye. Therefore, it is a much faster and easier method to determine bacterial growth than the common agar plate counting. In addition, it is possible to incorporate antimicrobial agents into the chromatic matrix, which enables the simultaneous detection of antibiotic susceptibility while monitoring bacterial growth. Such a measurement can also be performed at the single-cell level, as a single cell can be fluorescently detected. This method has been proven suitable for various *E. coli* strains, *Bacillus cereus* and *S. enterica*, and is potentially applicable to a wide range of bacteria, including Gram-positive and Gram-negative strains. One major disadvantage of this technique is that it does not differentiate between bacterial species and is not suitable for screening unknown pathogens. Bacterial mixtures, common for clinical samples, cannot be analysed by this method. Furthermore, no official standardized protocol is available. Despite these disadvantages that require more research, the PDA sensor method is a fast screening alternative to the gold-standard agar plate counting method.

Flow cytometry and fluorescence-activated cell sorting (FACS)

Fluorescence-activated cell sorting (FACS), a method of flow cytometry, is used to distinguish between cell types and to determine their viability and count by light scattering and fluorescent activity. Bacterial viability and antibiotic susceptibility can be determined by combining bacteria, antibiotics and relevant fluorescent stains. After 2 hours of incubation of cells with fluorescent stains, the sample can be analysed by flow cytometry. Standardized protocols and standardized data interpretation are not available for AST and vary for each pathogen and antimicrobial agent. So far, flow cytometry in AST has primarily been used for MRSA, MSSA, *E. coli* and *S. enterica*. As certain fluorescent stains can bind to nucleic acids within the bacterial cells, permeabilized cells give stronger fluorescent emission than non-permeabilized cells, and completely lysed cells give an even greater amount of fluorescence. For example, MRSA were found to show a greater population of viable cells than MSSA after incubation with oxacillin. However, the amount of fluorescence activity is not necessarily in direct correlation with the viability of the cells, due to the fact that flow cytometry inadequately distinguishes single cells from cell aggregates. Second, the amount of fluorescence is dependent on size and metabolic characteristics that vary per bacterial strain, and are also dependent on the antibiotic used. Third, some antibiotics can alter the extent of permeability of bacteria by inducing a membrane potential. Cell lysis often occurs due to exposure to β-lactams and results in brighter fluorescent emission without a change in the number of cells. Flow cytometric results and images taken with an epifluorescence microscope should, therefore, be interpreted carefully. Shrestha et al. as well as Sánchez-Romero and Casadesús found that antibiotic resistance could be determined and distinguished from susceptible strains after 2 hours with both side and forward scatter using flow cytometry. Another benefit of this technique is that it can be used for prokaryotic and eukaryotic cells. With its fast performance and wide potential, flow cytometry could find powerful application in research and in clinical screening. However, extensive research is needed to improve its validity.

Image analysis software for quantification of bacterial growth

Bacterial growth can also be analysed by taking images of different growth stages and quantifying them with an image analysis program. There are different types of software available. These software programs automatically quantifies the size, granularity, colour and location of microbial organisms on solid agar plates. It includes a collection of image analysis algorithms that can detect low-density organisms from plate micrographs that are barely visible to the human eye. Most software programs for quantifications are open source, and when using only agar colonization, this method is relatively cheap, with easy handling and is applicable for a variety of pathogens. Due to their high sensitivity, image analysis software and digital plate reading systems are able to detect early growth of pathogens. MICs can, therefore, be determined much faster. Furthermore, the exposure to pathogens is decreased when closed systems are used and common laboratory work, such as plate labelling, is not necessary due to automation. Some disadvantages of image analysis software or digital plate reading systems are that co-culture with eukaryotic cells is not possible with agar plates, and antimicrobial release from a substrate other than solid agar is not available. In order to determine the exact MIC, many consecutive images have to be taken and analysed, which can still be time-consuming, depending on the software, number of samples and extent of automation.

Matrix-Assisted Laser Desorption / Ionisation Time-Of-Flight Mass Spectrometry (MALDI-TOF MS)

Introduced in 2005 as an AST method, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOFMS) is a mass spectrometric technique that uses soft ionisation by laser pulses for the analysis of biomolecules. Ions are accelerated in this process and their respective time of flight is measured, where the smallest travel the fastest (Fig. 1). Thereby, the mass-to-charge ratio of a variety of compounds is determined, and species and strains can be distinguished based on their ribosomal proteins. Analyte-specific spectra are produced that can be compared with databases for pathogen identification. This technique can distinguish between pathogens in blood and urine samples, as well as bacterial strains in a bacterial mixture within approximately 30 minutes. With these rapid processing times,

MALDI-TOF has been deployed for AST in the clinics. Even though acquisition costs for the equipment are high, operating costs per analysis are very low. It has, therefore, already been introduced in clinical microbiology laboratories. Furthermore, the system does not depend on metabolic reactions and can be used for Gram-positive and Gram-negative bacteria and yeast. MALDI-TOF MS, however, suffers from several disadvantages. Samples must be analyzed within 48 hours of collection; otherwise the peaks in the spectra are difficult to distinguish, plausibly due to ribosomal protein degradation. Automation of processes is advised, as most samples, except for urine, require various time-consuming preparation steps. Whereas the identification of pathogens is successful, strain identification to assess resistance remains an issue in the cases of limited difference in ribosomal sequences. Therefore, distinguishing resistant strains unequivocally from non-resistant ones is not possible. Results on the efficacy of MALDI-TOF to differentiate between MRSA and MSSA are contradictory. Overall, this relatively new method opens new possibilities for reliability and efficiency in AST, though further validation is required.

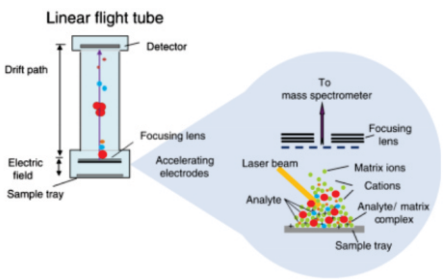


Fig. 1 Schematic representation of matrix-assisted laser desorption/ionisation time-offlight mass spectrometry (MALDI-TOF MS), in which soft ionisation of the microbial sample by laser pulses (right) results in ion displacement through the linear flight tube (left). Differences in flight time to the detector represent differences in ribosomal proteins.

Optical tweezers

In a proof-of-principle study, Samadi et al. showed that a single bacterium can be trapped in an optical tweezer, a 1064 nm laser beam, in order to assess the effect of chemical agents. The highly diluted sample is mounted on a piezoelectric transducer. The light scattered forward from the trapped cell is collected and sent to a position-sensitive detector. With statistical analysis of the position signal at different time points, the viability can be determined. This measurement is sensitive to such an extent that flagellar motion can be distinguished from the Brownian motion. Depending on the concentration of the chemical agent and bacteria type, the single-cell response can be determined within a couple of minutes, which is a major advantage. The single-cell trapping makes it a safe application for research and offers the opportunity for in-depth analysis of bacterial viability and the precise "killing time" upon antimicrobial exposure. Precise MICs can be determined without growing colonies or pre-culturing the cells. However, the system is only applicable for motile bacteria. For AST applications, this method has been demonstrated only for *E. coli* and, therefore, has no standardized protocols. With further research and validation, this technique could be promising due to its high-sensitivity capabilities.

Raman spectroscopy

Raman spectroscopy has also been demonstrated to be a precise technique to measure the susceptibility of bacteria to antimicrobial compounds. Using a frequency doubled argon ion laser at an excitation wavelength of 244 nm, Raman scattering of aromatic amino acids and nucleic acid bases is induced within bacterial cells and can be detected by vibrational bands. The total cell mass can be determined over time and plotted, giving the growth curve for the different bacterial growth phases, starting with the log phase, followed by the exponential growth phase, retardation phase and stationary phase prior to cell death. Every single phase can be seen within Raman spectra. Common analysis techniques include hierarchical cluster analysis (HCA) and principal component analysis (PCA). Raman spectroscopy can provide information about the underlying mechanism by which a drug affects the bacterial cells with the help of statistical methods. A different Raman technique, called the laser tweezers Raman spectroscopy (LTRS), allows single-cell analysis. In contrast to the common Raman technique, LTRS is applicable to low bacterial concentrations and distinguishes cellular drug response from normal growth of *E. coli* after 4.5 hours. Raman spectroscopy has the advantage that any type of microbe and antimicrobial compound can be analysed, as long as nucleic acids and proteins are present to a sufficient extent, and size and migration characteristics are taken into account. Additionally, Raman spectroscopy is highly sensitive regarding which bands of nucleic acids and which building blocks of proteins are excited. This makes it possible to assess the actual mechanism of antimicrobial compounds to kill microbes. One disadvantage is the high costs of Raman spectroscopes, although running costs are relatively low. Even though Raman spectroscopy has been used in clinics as a diagnostic tool for cancer, it has not yet been applied as AST method.

Discussion

The first antimicrobial susceptibility methods on the market were various agar or broth dilution and disc diffusion techniques, which have become the gold-standard tests to which all other AST methods are currently compared during development, identification, validation and clinical trials. Despite their simplicity, gold-standard techniques are still clinically applied with almost no changes since the first development, as they possess the major benefits of being cheap and not requiring any expensive equipment. Furthermore, as these techniques have been applied extensively, they are found to be very reliable in determining the antimicrobial effectivity and have been validated for a variety of bacterial strains. However, such techniques fail to provide insight into the mechanism of antimicrobial resistance, are relatively time consuming and require knowledge of working with the specific bacteria.

Conclusion

Currently, microbial infections have become an important clinical threat, with significant associated morbidity and mortality which is mainly due to the development of microbial resistance to the existing antimicrobial agents. Therefore, methods for anti-microbial susceptibility testing and discovering novel anti-microbial agents have been extensively used and continue to be developed. Some techniques were subjected to standardization by the CLSI and EUCAST, marking the major remarkable steps on this field.

Conventional AST has its diagnostic limitations it is generally time-consuming, and actionable results have a tendency for late arrival. The current methods are very solid and well-respected and do generally have CE and FDA certification. Any new technology has to compete with current reference standards, and the method that shows significant improvements has yet to be published. As we described here, several technologies may be knocking on the door shortly.

Automated Endoscope Reprocessors (Issue II)

6.5 Cleaning and Disinfection

6.5.1 Automated versus manual reprocessing of flexible endoscopes

RECOMMENDATION

EWDs compliant with EN ISO 15883 standard series should be the first choice for endoscope cleaning and disinfection, in order to:

- Provide a standardized and validated reprocessing cycle in a closed environment
- Document the process steps automatically (via a printer or electronically)
- Provide reliable and reproducible reprocessing
- Minimize staff contact with chemicals and contaminated equipment
- Minimize contamination of the environment
- Facilitate the work involved for personnel
- Lower the risk of damage to endoscopes

The process set-up in an EWD is standardized and allows automated documentation of all critical process parameters.(See Table 2 for the advantages and disadvantages of EWDs). Documentation and traceability are important for verification of reprocessing quality and to achieve the highest possible EWD level of safety for patients.

Manual reprocessing may also give reliable results, if staff perform the reprocessing conscientiously, according to defined standard operating procedures. These procedures should be controlled and documented in order to verify the process.

Manual reprocessing is more difficult to standardize and prone to human error and the risk of recontamination. Moreover, staff may have increased exposure to chemicals and infectious material.

Table 2 Advantages and disadvantages of endoscope washer-disinfectors (EWDs)

Advantages

- High level of standardization in reprocessing
- Low infection risk for patients and staff
- Complete documentation
- Full compatibility with latest European norms
- Economical use of chemicals and other resources
- User-friendly
- Reliable
- Less workload compared to full manual reprocessing
- Validation of full process for increased reliability

Disadvantages

- Potentially high costs
- Requires dedicated user skills/knowledge; more complex and more training required
- Additional validation costs to be covered by users
- Risk of infection if not regularly maintained
- If EWD breaks down, endoscopy procedure may have to be cancelled

6.5.2Cleaning and Disinfection in EWDs

RECOMMENDATION

EWDs compliant with the EN ISO 15883 standard series should be used for endoscope reprocessing.

RECOMMENDATION

After completion of bedside and manual cleaning, endoscopes and their components should be placed correctly in the EWD.

RECOMMENDATION

All endoscope channels should be connected to the EWD according to the manufacturer's IFU, even if they have not been used during the patient procedure.

The EN ISO 15883 standard series provides specifications and requirements for EWDs. This standard has enabled ESGE and ESGENA as well as European countries (Netherlands, Germany, Austria, UK) to prepare guidelines on validation.

However, if EWDs are not maintained appropriately, they may themselves become an infection risk by contamination of endoscopes during reprocessing. Regular maintenance and validation of reprocessing cycles is mandatory in order to ensure safe performance under the specifications of the EWD.

In addition to cleaning, disinfection, rinsing steps, and self-disinfection,the following features of an EWD may be helpful:

- Leak testing.
- Means for providing water of the required microbiological quality.
- Automatic air purging.
- Drying function.
- Detection of channel obstruction.
- Channel non-connection testing.
- Elements for providing and maintaining required temperature throughout the cleaning and disinfection steps.
- Means for documentation of cycle parameters, and identification of the endoscope and the operator.

RECOMMENDATION

All users of EWDs should be trained prior to first use. Regular training updates should be considered, and all training should be documented by the clinical service provider.

The distributor or company installing the EWD should carry out detailed training of every user. At a minimum, the training should cover:

- The EWD settings.
- Correct loading and unloading of endoscopes.
- Correct adaptation/use of connectors.
- User troubleshooting activities required in case of errors.
- EWD maintenance (relevant for daily, weekly, or monthly checks).

RECOMMENDATION

Manual reprocessing procedures should be in place incase of malfunctioning or defects.

Staff must be trained in manual reprocessing procedures. Additional access to EWDs in neighboring units may also be an option, provided that access and compatibility has been proven.

6.5.3 Disinfection in automated disinfection devices (ADDs)
RECOMMENDATION
Wherever possible, EWDs complying with EN ISO 15883standard series, should be used. If ADDs are used, they should at least comply with the relevant parts of the EN ISO 15883-4 standard.

The automated disinfection process does not usually have an integrated automated cleaning stage. ADDs are intended to disinfect flexible endoscopes in a closed system after complete and careful manual cleaning.
Some ADDs offer:
- Integrated leakage testing.
- Rinsing step.
- Air purge.

See Table 3 for advantages and disadvantages of ADDs

Table 3 Advantages and disadvantages of automated disinfection devices (ADDs).
Advantages
- Lower purchasing costs compared with EWDs
- Less workload compared with full manual reprocessing

Disadvantages
- Greater workload compared with EWDs
- No European standard available for design, type testing, performance requirements, and validation
- In the case of reuse of disinfectant, effective concentration must be confirmed, if applicable
- Increased workload of routine testing (i. e., disinfectant efficacy testing)
- Traceability and documentation activities are more time-consuming
- More complex; more training required
- Risk of infection if not regularly maintained

References:
Herrin A, Loyola M, Bocian S et al. Society of Gastroenterology Nurses and Associates (SGNA) Practice Committee. Standards of infection prevention in the gastroenterology setting 2015. https://www.sgna.org/Portals/0/Education/PDF/Standards-Guidelines/Standard%20of% 20Infection%20Prevention_FINAL.pdf
Gutterman E, Horgensen L, Mitchell A et al. Adverse staff health outcomes associated with endoscope reprocessing. Biomed Instrum Technol 2013: 172 – 179
Drysdale SA. The incidence of upper extremity injuries in endoscopy nurses working in the United States. Gastroenterol Nurs 2013; 36:329 – 338
Beilenhoff U, Brljak J, Neumann C et al. European Society of Gastroenterology Nurses and Associates (ESGENA). ESGENA Position Statementon health and safety in GI endoscopy. [In press] www.esgena.org
Hugonnet S, Chevrolet JC, Pittet D. The effect of workload on infection risk in critically ill patients. Crit Care Med 2007; 35: 76 – 81
Erasmus V, Daha TJ, Brug H et al. Systematic review of studies on compliance with hand hygiene guidelines in hospital care. Infect Control Hosp Epidemiol 2010; 31: 283 – 294
Santos LY, Souza Dias MB, Borrasca VL et al. Improving hand hygiene adherence in an endoscopy unit. Endoscopy 2013; 45: 421– 425
Ofstead CL, Wetzler HP, Snyder AK et al. Endoscope reprocessing methods: a prospective study on the impact of human factors and automation. Gastroenterol Nurs 2010; 33: 304 – 311
Aumeran C, Poincloux L, Souweine B et al. Multidrug-resistant Klebsiella pneumoniae outbreak after endoscopic retrograde cholangiopancreatography. Endoscopy 2010; 42: 895 – 899
Beilenhoff U, Brljak J, Neumann C. European Society of Gastroenterology Nurses and Associates (ESGENA). et al. European curriculum for reprocessing of flexible endoscopes 2018. [In press] www.esgena.org
European Society of Gastroenterology Nurses and Associates(ESGENA). European job profile for endoscopy nurses. Endoscopy2004; 36: 1025 – 1030
European Council. Council Directive 93/42/EEC of 14 June 1993 concerning medical devices. Official Journal L169, 12/07/1993 P0001–0043. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31993L0042
Costa DM, Lopes LKO, Hu H et al. Alcohol fixation of bacteria to surgical instruments increases cleaning difficulty and may contribute to sterilization inefficacy. Am J Infect Control 2017; 45: e81 – e86
International Organization for Standardization. ISO 15883–4: Washer-disinfectors – Part 4: Requirements and tests for washer-disinfectorsemploying chemical disinfection for thermolabile endoscopes.2008: Available at: https://www.iso.org/standard/63696.html
Rutala WA, Weber DJ. Gastrointestinal endoscopes: a need to shift from disinfection to sterilization? JAMA 2014; 312: 1405 – 1406 [45] Roberts CG. The role of biofilms in reprocessing medical devices. Am J Infect Control 2013; 41: 577 – 580
Otter JA, Vickery K, Walker JT et al. Surface-attached cells, biofilm and biocide susceptibility: implications for hospital cleaning and disinfection. J Hosp Infect 2015; 89: 16 – 27
Gastroenterological Society of Australia/Gastroenterological Nurses College of Australia (GESA/GENCA). Draft document for discussion. Infection control in endoscopy – Multidrug resistant outbreaks response.04 2016: Available from: http://www.genca.org/public/5/files/Infection%20Control%20Guidelines%20Draft%20April%202016.pdf

To be continued……

Barbara McClintock

Barbara McClintock (June 16, 1902 – September 2, 1992) was an American scientist and cytogeneticist who were awarded the 1983 Nobel Prize in Physiology or Medicine. McClintock received her PhD in botany from Cornell University in 1927. There she started her career as the leader in the development of maize cytogenetics, the focus of her research for the rest of her life. From the late 1920s, McClintock studied chromosomes and how they change during reproduction in maize. She developed the technique for visualizing maize chromosomes and used microscopic analysis to demonstrate many fundamental genetic ideas. One of those ideas was the notion of genetic recombination by crossing-over during meiosis—a mechanism by which chromosomes exchange information. She produced the first genetic map for maize, linking regions of the chromosome to physical traits. She demonstrated the role of the telomere and centromere, regions of the chromosome that are important in the conservation of genetic information. She was recognized as among the best in the field, awarded prestigious fellowships, and elected a member of the National Academy of Sciences in 1944.

McClintock began her studies at Cornell's College of Agriculture in 1919. There, she participated in student government and was invited to join a sorority, though she soon realized that she preferred not to join formal organizations. Instead, McClintock took up music, specifically jazz. She studied botany, receiving a BSc in 1923.Her interest in genetics began when she took her first course in that field in 1921. The course was based on a similar one offered at Harvard University, and was taught by C. B. Hutchison, a plant breeder and geneticist

During her time at Missouri, McClintock expanded her research on the effect of X-rays on maize cytogenetics. McClintock observed the breakage and fusion of chromosomes in irradiated maize cells. She was also able to show that, in some plants, spontaneous chromosome breakage occurred in the cells of the endosperm. Over the course of mitosis, she observed that the ends of broken chromatids were rejoined after the chromosome replication. In the anaphase of mitosis, the broken chromosomes formed a chromatid bridge, which was broken when the chromatids moved towards the cell poles. The broken ends were rejoined in the interphase of the next mitosis, and the cycle was repeated, causing massive mutation, which she could detect as variegation in the endosperm. This - breakage rejoining bridge cycle was a key cytogenetic discovery for several reasons. First, it showed that the rejoining of chromosomes was not a random event, and second, it demonstrated a source of large-scale mutation. For this reason, it remains an area of interest in cancer research today.

Discovery of controlling elements

In the summer of 1944 at Cold Spring Harbor Laboratory, McClintock began systematic studies on the mechanisms of the mosaic color patterns of maize seed and the unstable inheritance of this mosaicism. She identified two new dominant and interacting genetic loci that she named Dissociation (Ds) and Activator (Ac). She found that the Dissociation did not just dissociate or cause the chromosome to break, it also had a variety of effects on neighbouring genes when the Activator was also present, which included making certain stable mutations unstable. In early 1948, she made the surprising discovery that both Dissociation and Activator could transpose, or change position, on the chromosome.

She observed the effects of the transposition of Ac and Ds by the changing patterns of coloration in maize kernels over generations of controlled crosses, and described the relationship between the two loci through intricate microscopic analysis. She concluded that Ac controls the transposition of the Ds from chromosome 9, and that the movement of Ds is accompanied by the breakage of the chromosome. When Ds moves, the aleurone-color gene is released from the suppressing effect of the Ds and transformed into the active form, which initiates the pigment synthesis in cells. The transposition of Ds in different cells is random, it may move in some but not others, which causes color mosaicism. The size of the colored spot on the seed is determined by stage of the seed development during dissociation. McClintock also found that the transposition of Ds is determined by the number of Accopies in the cell.

In 1957, McClintock received funding from the National Academy of Sciences to start research on indigenous strains of maize in Central America and South America. She was interested in studying the evolution of maize through chromosomal changes, and being in South America would allow her to work on a larger scale. McClintock explored the chromosomal, morphological, and evolutionary characteristics of various races of maize. After extensive work

in the 1960s and 1970s, McClintock and her collaborators published the seminal study The Chromosomal Constitution of Races of Maize, leaving their mark on paleobotany, ethnobotany, and evolutionary biology.

McClintock officially retired from her position at the Carnegie Institution in 1967,and was made a Distinguished Service Member of the Carnegie Institution of Washington. This honor allowed her to continue working with graduate students and colleagues in the Cold Spring Harbor Laboratory as scientist emerita; McClintock was widely credited with discovering transposition after other researchers finally discovered the process in bacteria, yeast, and bacteriophages in the late 1960s and early 1970s.During this period, molecular biology had developed significant new technology, and scientists were able to show the molecular basis for transposition. In the 1970s, Ac and Ds were cloned by other scientists and were shown to be class II transposons. Ac is a complete transposon that can produce a functional transposase, which is required for the element to move within the genome. Ds has a mutation in its transposase gene, which means that it cannot move without another source of transposase. Thus, as McClintock observed, Ds cannot move in the absence of Ac. Spm has also been characterized as a transposon. Subsequent research has shown that transposons typically do not move unless the cell is placed under stress, such as by irradiation or the breakage-fusion-bridge cycle, and thus their activation during stress can serve as a source of genetic variation for evolution. McClintock understood the role of transposons in evolution and genome change well before other researchers grasped the concept. Nowadays, Ac/Ds is used as a tool in plant biology to generate mutant plants used for the characterization of gene function.

HONORS AND RECOGNITION

In 1947, McClintock received the Achievement Award from the American Association of University Women. She was elected a Fellow of the American Academy of Arts and Sciences in 1959.In 1967, McClintock was awarded the Kimber Genetics Award; three years later, she was given the National Medal of Science by Richard Nixon in 1970. She was the first woman to be awarded the National Medal of Science. Cold Spring Harbor named a building in her honor in 1973.She received the Louis and Bert Freedman Foundation Award and the Lewis S. Rosensteil Award in 1978.In 1981, she became the first recipient of the MacArthur Foundation Grant, and was awarded the Albert Lasker Award for Basic Medical Research, the Wolf Prize in Medicine and the Thomas Hunt Morgan Medal by the Genetics Society of America. In 1982, she was awarded the Louisa Gross Horwitz Prize from Columbia University for her research in the "evolution of genetic information and the control of its expression.

Most notably, she received the Nobel Prize for Physiology or Medicine in 1983, the first woman to win that prize unshared, and the first American woman to win any unshared Nobel Prize. It was given to her by the Nobel Foundation for discovering "mobile genetic elements"; this was more than 30 years after she initially described the phenomenon of controlling elements. She was compared to Gregor Mendel in terms of her scientific career by the Swedish Academy of Sciences when she was awarded the Prize.

McClintock spent her later years, post Nobel Prize, as a key leader and researcher in the field at Cold Spring Harbor Laboratory on Long Island, New York. McClintock died of natural causes in Huntington, New York, on September 2, 1992 at the age of 90; she never married or had children.

Jokes

An Investment Banker Was Getting Married.
During Wedding, The Wife Vomits.
Husband: "What Happened?"
Wife: "Capital Gains Arising Out Of Previous Investment."
Husband: "U cheated me.."
Wife: "U should know, mutual fund investments are subject to market risks!"

Santa Had A Leakage In The Roof Over His Dining Room.
Plumber Asked: "Sir When Did U Notice Leakage in Roof ?"
Santa: "Last Night.... When It Took Me 3 Hours To Finish My Delicious Chicken Soup"

HR Manager Asked Banta in an Interview.
"Can you spell a word that has more than 75 Letters in it?"
Banta confidently Replied: "Letter Box"

Once in a airoplane 5 Person Travelling-
Dhoni, Ambani, Rahul Gandhi, Narendra Modi and a little child.
Suddenly there was some defect in the plane.
There were four parachutes on there.
Dhoni said: I am the world's great batsman, I have to stay alive, then he jumped with a parachute
Ambani said: I am in the rich people of India
I have to stay alive, then he jumped with a parachute.
Rahul Gandhi said: I am the most popular leader in this country
I have to stay alive. then he jumped with a parachute.
Narendra Modi speaks to the child: Son you are future of country go to the last parachute Take the jump.
Child Speech: But here are two parachutes, Rahul Gandhi jumped with school bag

A man noticed his credit card has been stolen - but he never reported it. Do You Know Why?
That thief was still spending considerably very less than his wife.

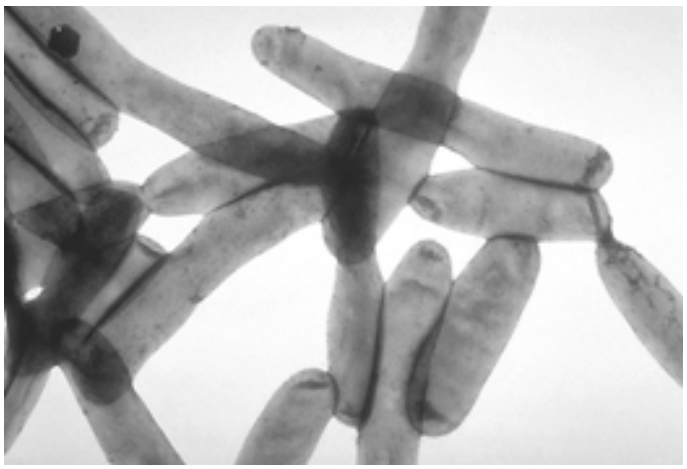
1 property dealer gives an ad for Lake View Flats in Kolkata.
When pappu bought that Flat he found something else instead of Lake View.
Property dealer called to Pappu to change Flat.
Pappu said - I do want to change Flats.
Infact there is College Girls Hotel View in place of Lake View.

During a job interview :
Boss : What's the highest level of education you obtained?
Candidate : PHD
Boss : Great! So that means you have a Doctor degree ...
Candidate : Welll! No... That means
Passed Highschool with Difficulties (P.H.D.)

A man went to Renown lawyer and told him,
"My neighbor owes me 50,000 Rupees and he won't pay up. What should I do?"
"Do you have any proof he owes you the money?" asked the lawyer. "No," replied the man.
"OK, then write him a letter asking him for the 500000 Rupees he owed you," said the lawyer.
"But it's only 50,000," replied the man. "Precisely. That's what he will reply and then you'll have your proof!"

Two Commerce Students talking...
Appu : Oh ! Sorry yaar, I heard about your breakup. Is it true?
Pappu: Yup!
Appu : You must be sad na.. ....
Pappu : No yaar ! We are commerce students! I have kept 1 Girlfriend as reserved for doubtful debts.

Legionella pneumophila



Legionella pneumophila is a thin, aerobic, pleomorphic, flagellated, non-spore-forming, Gram-negative bacterium of the genus Legionella. *L. pneumophila* is the primary human pathogenic bacterium in this group and is the causative agent of Legionnaires' disease, also known as legionellosis.

L. pneumophila is a Gram-negative, none capsulated, aerobic bacillus with a single, polar flagellum often characterized as being a coccobacillus. It is aerobic and unable to hydrolyse gelatin or produce urease. It is also nonfermentative. *L. pneumophila* is neither pigmented nor does it autofluoresce. It is oxidase- and catalase-positive, and produces beta-lactamase. *L. pneumophila* colony morphology is gray-white with a textured, cut-glass appearance; it also requires cysteine and iron to thrive. It grows on yeast extract in "opal-like" colonies.

While *L. pneumophila* is categorized as a Gram-negative organism, it stains poorly due to its unique lipopolysaccharide content in the outer leaflet of the outer cell membrane. The bases for the somatic antigen specificity of this organism are located on the side chains of its cell wall. The chemical composition of these side chains both with respect to components and arrangement of the different sugars, determines the nature of the somatic or O-antigen determinants, which are important means of serologically classifying many Gram-negative bacteria. At least 35 different serovars of *L. pneumophila* have been described, as well as several other species being subdivided into a number of serovars.

PATHOGENESIS

In humans, *L. pneumophila* invades and replicates inside macrophages. The internalization of the bacteria can be enhanced by the presence of antibody and complement, but is not absolutely required. Internalization of the bacteria appears to occur through phagocytosis. However, *L. pneumophila* is also capable of infecting nonphagocytic cells through an unknown mechanism. A rare form of phagocytosis known as coiling phagocytosis has been described for *L. pneumophila*, but this is not dependent on the Dot/Icm secretion system and has been observed for other pathogens. Once internalized, the bacteria surround themselves in a membrane-bound vacuole that does not fuse with lysosomes that would otherwise degrade the bacteria. In this protected compartment, the bacteria multiply.

The bacteria use a type IVB secretion system known as Dot/Icm to inject effector proteins into the host. These effectors are involved in increasing the bacteria's ability to survive inside the host cell. *L. pneumophila* encodes for over 330 "effector" proteins, which are secreted by the Dot/Icm translocation system to interfere with host cell processes to aid bacterial survival. It has been predicted that the genus *Legionella* encodes more than 10,000 and possibly up to ~18,000 effectors that have a high probability to be secreted into their host cells. One key way in which *L. pneumophila* uses its effector proteins is to interfere with fusion of the *Legionella*-containing vacuole with the host's endosomes, and thus protect against lysis. Knock-out studies of Dot/Icm translocated effectors indicate that they are vital for the intracellular survival of the bacterium, but many individual effector proteins are thought to function redundantly, in that single-effector knock-outs rarely impede intracellular survival. This high number of translocated effector proteins and their redundancy is likely a result of the bacterium having evolved in many different protozoan hosts.

For *Legionella* to survive within macrophages and protozoa, it must create a specialized compartment known as the *Legionella*-containing vacuole (LCV). Through the action of the Dot/Icm secretion system, the bacteria are able to prevent degradation by the normal endosomal trafficking pathway and instead replicate. Shortly after internalization, the bacteria specifically recruit endoplasmic reticulum-derived vesicles and mitochondria to the LCV while preventing the recruitment of endosomal markers such as Rab5 and Rab7. Formation and maintenance of the vacuoles are crucial for pathogenesis; bacteria lacking the Dot/Icm secretion system are not pathogenic and cannot replicate within cells, while deletion of the Dot/Icm effector SdhA results in destabilization of the vacuolar membrane and no bacterial replication.

DIAGNOSIS

People of any age may suffer from Legionnaires' disease, but the illness most often affects middle-aged and older persons, particularly those who smoke cigarettes or have chronic lung disease. Immunocompromised people are also at higher risk. Pontiac fever most commonly occurs in persons who are otherwise healthy.

The most useful diagnostic tests detect the bacteria in coughed-up mucus, find *Legionella* antigens in urine samples, or allow comparison of *Legionella* antibody levels in two blood samples taken 3–6 weeks apart. A urine antigen test is simple, quick, and very reliable, but only detects *L. pneumophila* serogroup 1, which accounts for 70% of disease caused by *L. pneumophila*, which means use of the urine antigen test alone may miss as many as 30% of cases. This test was developed by Richard Kohler in 1982. When dealing with *L. pneumophila* serogroup 1, the urine antigen test is useful for early detection of Legionnaire's disease and initiation of treatment, and has been helpful in early detection of outbreaks. However, it does not identify the specific subtypes, so it cannot be used to match the person with the environmental source of infection. The *Legionella* bacteria can be cultured from sputum or other respiratory samples. *Legionella* spp. stain poorly with Gram stain, stains positive with silver, and is cultured on

charcoal yeast extract with iron and cysteine (CYE agar).

A significant under-reporting problem occurs with legionellosis. Even in countries with effective health services and readily available diagnostic testing, about 90% of cases of Legionnaires' disease are missed. This is partly due to the disease being a relatively rare form of pneumonia, which many clinicians may not have encountered before, thus may misdiagnose. A further issue is that people with legionellosis can present with a wide range of symptoms, some of which (such as diarrhea) may distract clinicians from making a correct diagnosis.

TREATMENT

Effective antibiotics include most macrolides, tetracyclines, ketolides, and quinolones. *Legionella* spp. multiply within the cell, so any effective treatment must have excellent intracellular penetration. Current treatments of choice are the respiratory tract quinolones (levofloxacin, moxifloxacin, gemifloxacin) or newer macrolides (azithromycin, clarithromycin, roxithromycin). The antibiotics used most frequently have been levofloxacin, doxycycline, and azithromycin.

Macrolides (azithromycin) are used in all age groups, while tetracyclines (doxycycline) are prescribed for children above the age of 12 and quinolones (levofloxacin) above the age of 18. Rifampicin can be used in combination with a quinolone or macrolide. Whether rifampicin is an effective antibiotic to take for treatment is uncertain. The Infectious Diseases Society of America does not recommend the use of rifampicin with added regimens. Tetracycline and erythromycin led to improved outcomes compared to other antibiotics in the original American Legion outbreak. These antibiotics are effective because they have excellent intracellular penetration in *Legionella*-infected cells. The recommended treatment is 5–10 days of levofloxacin or 3–5 days of azithromycin, but in people who are immunocompromised, have severe disease, or other pre-existing health conditions, longer antibiotic use may be necessary. During outbreaks, prophylactic antibiotics have been used to prevent Legionnaires' disease in high-risk individuals who have possibly been exposed.

The mortality at the original American Legion convention in 1976 was high (29 deaths in 182 infected individuals) because the antibiotics used (including penicillins, cephalosporins, and aminoglycosides) had poor intracellular penetration. Mortality has plunged to less than 5% if therapy is started quickly. Delay in giving the appropriate antibiotic leads to higher mortality.

Elevated estrogen levels in the womb linked to autism

Elevated latent prenatal steroidogenic activity has been found in the amniotic fluid of autistic boys, based on measuring prenatal androgens and other steroid hormones. To date, it is unclear if other prenatal steroids also contribute to autism likelihood. Prenatal oestrogens need to be investigated, as they play a key role in synaptogenesis and corticogenesis during prenatal development, in both males and females. Here we test whether levels of prenatal oestriol, oestradiol, oestrone and oestrone sulphate in amniotic fluid are associated with autism, in the same Danish Historic Birth Cohort, in which prenatal androgens were measured, using univariate logistic regression ($n = 98$ cases, $n = 177$ controls). We also make a like-to-like comparison between the prenatal oestrogens and androgens. Oestradiol, oestrone, oestriol and progesterone each related to autism in univariate analyses after correction with false discovery rate. A comparison of standardised odds ratios showed that oestradiol, oestrone and progesterone had the largest effects on autism likelihood. These results for the first time show that prenatal oestrogens contribute to autism likelihood, extending the finding of elevated prenatal steroidogenic activity in autism. This likely affects sexual differentiation, brain development and function.

The male-biased prevalence of autism, together with the finding that autistic girls have a higher mutational load than autistic boys, suggests that males have a higher likelihood of developing autism. The sex ratio in autism diagnoses persists even after taking into account under- and/or mis-diagnosis, as well as camouflaging in females, with males being three times more likely to have the condition. This implicates mechanisms of sexual differentiation in the development of autism. Five recent findings support this inference.

First, autistic women have atypical brain structure in sexually dimorphic regions, when assessed via magnetic resonance imaging and compared to neurotypical controls. Second, functional connectivity in the brain of males with autism shows both hypermasculine and hyperfeminine patterns, when assessed in relation to neurotypical sex differences. Third, autistic people show a masculinised shift in scores on two key sexually dimorphic psychological traits, empathy and systemising, a finding that has been replicated in a big data study of 36,000 autistic people. Fourth, autistic women have elevated androstenedione levels, the precursor to testosterone. Finally, fifth, autistic children have hypermasculine facial features, as rated using three-dimensional photogrammetry.

Although autism is strongly heritable and sex-associated genetic mechanisms could contribute to this implication of sexual differentiation in autism, prenatal hormone exposure and a brief surge in foetal testosterone are critical for sexual differentiation and masculinisation in human. In line with this, we previously found elevated steroidogenic activity during this prenatal masculinisation window (PMW) in the amniotic fluid of autistic boys. Subsequently, three very large epidemiological studies revealed a link between autism and maternal polycystic ovarian syndrome (PCOS), a condition associated with androgenic excess. Consistently with this, the 2D:4D digit ratio, a marker of prenatal androgen exposure, is also masculinised in autistic children and their parents. Finally, autistic women and their mothers have elevated rates of steroid-related cancers, such as breast cancer and ovarian cancer.

However, a number of studies that focused on testosterone have not replicated the correlation of hormonal levels with autistic traits. First, umbilical cord testosterone measured soon after birth was not associated with the development of autistic traits. Second, salivary testosterone during a brief period of postnatal steroid surge ('mini-puberty') also did not correlate with autistic traits in toddlers. In both cases, testosterone was measured postnatally—in the neonatal period—rather than during the PMW, during which foetal testosterone is first produced and masculinisation of the brain and body commences. This would suggest that timing is critical for the effects of testosterone on the brain, with the late first-early second trimester PMW being key, rather than the neonatal period. Finally, univariate assessment of amniotic testosterone in a separate cohort of neurotypical children also failed to reveal an association to autistic traits in childhood . This latter finding may reflect that the wider endocrine environment outside testosterone is also significant for autism likelihood.

While prenatal androgens are responsible for masculinisation in humans, prenatal oestrogens also contribute to foetal and neonatal brain development and yet these have not been thoroughly investigated for their potential role in autism likelihood. Oestrogens and their receptors are widespread in the developing brain in both males and females and regulate many neurodevelopmental processes, including synaptogenesis, apoptosis and neuronal differentiation. Oestradiol in particular supports synapse formation in the cortex by enhancing excitatory GABA activity. In autism, synapse formations, neuronal differentiation as well as the GABAergic system are all atypical. These provide clues that prenatal oestrogens may be involved in autism. However, we still lack direct evidence of this.

With regard to clinical studies in humans, low oestriol in maternal serum during the second trimester of pregnancy significantly increases the likelihood of autism in the foetus, as demonstrated in a large study of $n = 2586$ autistic pregnancies. This study may have been confounded by a variety of pregnancy complications, such as pre-eclampsia and being small for gestational age, since these are also more frequent in autism. Thus, further study of prenatal oestrogenic activity, particularly in foetal circulation, is warranted. In addition, there is a need to compare different prenatal oestrogens to each other, in relation to autism likelihood.

In the present study, we measured prenatal levels of oestriol, oestradiol, oestrone and oestrone sulphate in amniotic fluid of boys with and without autism ($n = 98$ and $n = 177$ respectively) from the Danish Historic Birth Cohort (HBC), in the same samples in which we had found an elevated steroidogenic factor, following principal component analysis of prenatal androgens and other steroid hormones. We have expanded on these findings by assaying oestrogens and by assessments of each steroid hormone to autism likelihood via univariate logistic regression. We also investigated potential differences in the aromatising capacity in autism by comparing the ratio between androgens and oestrogens. Finally, we calculated standardised effect sizes for all hormones assayed to date in this cohort, in order to understand which amniotic fluid hormones make the largest contribution to autism likelihood.

Best Practices in Wound Care Management (Issue II)

Comprehensive Wound Assessment

A comprehensive wound assessment must consider and document the following aspects:

- Underlying cause
- Wound location and size
- Comorbidities
- Nutritional status of the patient
- Smoking habits
- Drug/alcohol use
- Mobility of the patient
- Circulation
- Infection
- Inflammation
- Odour
- Exudate
- Medication
- Site and type of pain, changes in nature of onset-triggers of pain
- Colour
- Periwound skin
- Wound bed
- Patient-centred concerns
- Patient's psychological status

Aetiological factors and comorbidities

Chronic medical conditions can continually erode the immune system, predisposing patients to complications simultaneously affecting several organs of the body, including the eye, blood vessels, kidneys and the nervous system (Ahmed, 2005).Immunosuppression with increased bacterial virulence can make wound infection more likely (Wounds UK, 2010) and play a significant part in chronicity.

- **Diabetes.** Metabolic disorders associated with diabetes impair immune and inflammatory cells (Falanga, 2000),increasing the risk of wound infection and decreasing the potential for wound healing. Saad et al (2013) stated that neuropathy, peripheral vascular disease and minor trauma could contribute to impaired healing in diabetic foot ulcers, with Novak (2010) warning that intermittent claudication, absent pedalpulses and ischaemic gangrene were more prevalent in patients with diabetes. Diabetes in the presence of elevated blood glucose will further reduce neutrophil activity and interfere with the action of phagocytosis, thus delaying the normal inflammatory response. In addition, associated peripheral neuropathy will mask indicators of wound infection such as inflammation, pain and discomfort (Jones, 2012). Regular inspection of these wounds is paramount.
- **Circulatory disorders.** Oxygen is essential for cell metabolism and critical to all wound-healing processes. It prevents wound infection, induces angiogenesis, increases keratinocytes, enhances fibroblast proliferation and collagen synthesis, and promotes wound contraction (Bishop, 2008; Rodriguez etal, 2008). Systemic conditions such as ageing, diabetes and atherosclerosis can impair vascular flow, setting the stage for poor tissue oxygenation, increased infection risk and delayed healing (Guoand Dipietro, 2010). Poor tissue perfusion due to ischemia also might lower infection resistance. Clinicians should consider using topical antimicrobialsin arterially compromised patients who have non-healing wounds, as reduced blood flow hinders cell, nutrient and oxygen transport to the wound bed (Lipsky and Hoey, 2009).

Lifestyle factors

- **Alcohol consumption.** Wigston et al(2013) identified that alcohol significantly affects non-healing. Excess alcohol consumption inhibits the inflammatory response, and delays collagen and epithelial cell production, and blood vessel growth during the proliferative stage of wound healing (Radek et al,2009). Encourage patients to reduce alcohol consumption during wound healing.
- **Tobacco smoking.** Pharmacologically, smoking's influence on wound healing is multifaceted. The literature has identified smoking as a potential risk factor for wound infection due to delayed re-epithelialisation through nicotine-dependent downregulation of keratinocyte migration or from reduced monocyte and neutrophil oxidative burst activity, leading to a higher bacterial count in the wound bed (Kean, 2010).Smoking leads to tissue ischaemia due to its vasoconstrictive effect. It results in lower oxygen levels from preferential uptake of carbon monoxide, thereby limiting oxygen available for oxidative killing by white cells. Smoking impairs white blood cell migration, resulting in lower numbers of monocytes and macrophages in the wound bed, and reduces neutrophil activity, increasing the risk of wound infection and delayed healing (Ahn et al, 2008). Smoking reduces collagen production and deposition, and might also delay healing, mainly due to its immunosuppressive action (Sørensen et al, 2009). In addition, smokers exhibit delayed epithelisation, resulting in a dampened white cell and inflammatory response, which results in a higher bacterial count in the wound bed (Jones, 2012).
- **Nutrition.** Malnourished patients have higher risk of infection and often experience chronic non-healing wounds with decreased tensile strength (Stechmiller, 2010).

Medications

Certain drugs that are vital to a patient's health status negatively affect the wound healing process. In all cases, liaise with the prescriber to analyse risks and benefits before stopping prescriptions.

- **Antibiotics.** Although antibiotic therapy is sometimes necessary to treat wound infection, these drugs should be used only in clinically infected wounds (Karukonda et al, 2000b) to encourage wound healing. However, antibiotics might also reduce the wound's tensile strength, impeding final wound closure(Diehr et al, 2007).
- **Anticancer drug.** Oncology drugs also negatively affect wound healing (Valls et al, 2009), but cessation is not advisable, so it is important that both the patient and the wound be carefully monitored and reassessed in a timely

manner. Chemotherapeutic drugs inhibit cellular metabolism, cell division and angiogenesis and, therefore, inhibit many of wound repair's critical pathways (Guoand Dipietro, 2010). In addition, they weaken the patient's immune functions, thereby impeding the inflammatory phase of wound healing and increasing the risk of wound infection.

- **Antiplatelet drugs.** Certain antiplatelet drugs have been found to hinder wound healing. Acetylsalicylic acid reduces platelet activation by preventing thrombus formation (Karukonda et al,2000a). Patients should refrain from taking these drugs unless doing so is essential.
- **Glucocorticoid steroids.** These anti-inflammatory agents inhibit wound repair and suppress cellular wound responses. However, they are also essential in some autoimmune disorders that lead to wounds. Systemic steroids cause incomplete granulation tissue and reduced wound contraction, resulting in hard-to-heal wounds (Franz et al,2007). Hydrocortisone and prednisolone stimulate the production of cortisol, which depresses the immune system, depleting either the neutrophils that move to the wound site or the concentration of the cytokines necessary for healing (Glaser et al, 1999).
- **NSAIDs.** Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to treat inflammation and pain. Short-term NSAID use does not adversely affect wound healing. However, long-term use might decrease fibroblast numbers, weaken skin strength, reduce wound contraction, delay epithelisation and impair angiogenesis (Dvivedi et al,1997; Jones et al, 1999).

The clinician assessing the patient and wound must understand the repercussions of comorbidities, lifestyle factors and medications on the wound. This knowledge will help ensure an appropriate topical antimicrobial treatment plan that's been tailored to the patient is implemented.

TIME

T – Tissue, non-viable or deficient
I – Infection / inflammation
M – Moisture imbalance
E – Edge of wound non-advancing or undermined

DEVELOPING A WOUND MANAGEMENT PLAN

The International Advisory Board on WoundBed Preparation developed a framework— known by the acronym TIME (see above) — to provide a means by which clinicians can approach optimising the wound bed. If infection or colonisation is clinically diagnosed, use TIME to develop a wound management plan that includes removing non-viable tissue, reducing oedema and exudate, reducing the bacterial burden and correcting any abnormalities to promote wound healing (Schultz et al, 2003;Falanga, 2004).

To be continued…….

References:

www.wounds-uk.com
Cutting KF, White RJ (2002) Maceration of the skin and wound bed. 1: Its nature and causes. J Wound Care 11(7):275-8.
Cutting K, White RJ, Maloney P, Harding KG(2005) Clinical identification of wound infection:
a Delphi approach. In: European Wound Management Association position document. Identifying criteria for wound infection. London:MEP Ltd.
Davies SC (2013) Annual Report of the Chief Medical Officer. Volume Two, 2011. Infections and the Rise of Antimicrobial Resistance. London: Department of Health. Available at: http://bit.ly/ZjYQLZ (accessed 12.06.2013)
Dealey C, Posnett J, Walker A (2012) The cost of pressure ulcers in the United Kingdom. J Wound Care 21(6)261–2.
Deloach ED, DiBenedetto RJ, Womble L, Gilley JD(1992) The treatment of osteomyelitis underlying pressure ulcers. Decubitus 5(6):32–41.

Highlights of the coming issue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