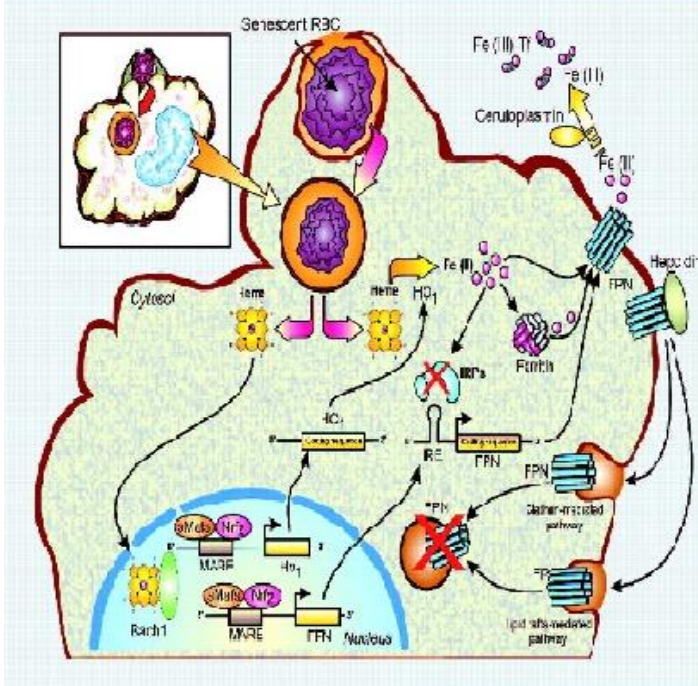
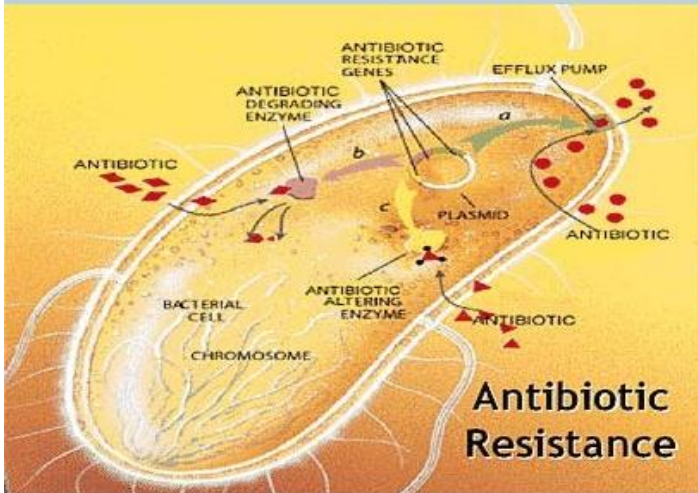
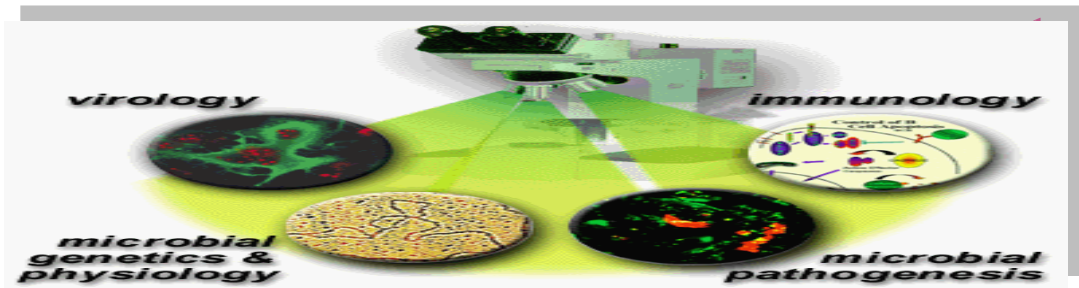


المجهر

مجلة علمية مهنية اجتماعية ثقافية
تصدر عن نقابة الطب المخبري الفلسطيني





Al-Mijhar

*The official Journal of
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Al-Mijhar:

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Contents:

- 1- LABORATORY ERRORS - SUSPECTED, REAL OR IMAGINED?..... 2.**
- 2- HEPCIDIN: The link to understanding iron regulation.....4.**
- 3- Reshaping healthcare at the clinical laboratory level.....12.**
- 4- The anarchy of antibiotic resistance: mechanisms of bacterial resistance19.**
- 5- CE Test30.**

LABORATORY ERRORS - SUSPECTED, REAL OR IMAGINED?

We strive to provide rapid, accurate laboratory data by continually implementing more accurate and precise methods and by improving our quality assurance techniques. In spite of these efforts, occasional errors will occur due to the human element involved and due to the inherent limitations of current methods.

You are the ultimate quality control for the laboratories and we suggest that you scan laboratory results with these questions in mind:

1. **Is the result reasonable?** (e.g., a serum potassium of 50 mEq/L in a living patient obviously is an error, probably due to a misplaced decimal point). If not, see step 4.
2. **Is the result consistent?** (a) **chronologically** with results on previous samples from the patient? (e.g., a report of BUN of 200 mg/dl in a patient with a normal BUN the previous day); (b) **internally** with other results on the same sample? (e.g., Do the electrolytes balance?) If not, see step 3.
3. **If the reported result is not consistent with the clinical findings, could the inconsistency be due to:**
 - a. **Collection technique?** (e.g., wrong anticoagulant used, prolonged tourniquet application, use of a hypodermic and syringe rather than vacutainer system which is more efficient and less likely to cause hemolysis, etc.)
 - b. **Physiologic variation?** (e.g., age, sex, pregnancy, posture, exercise, time of day, relationship to meals, etc.)
 - c. **Therapy?** (e.g., in vivo effect of drug on the test method itself).
Useful reference on drug laboratory interactions:
Effects of Drugs on Clinical Laboratory Tests, Donald S. Young, American Association for Clinical Chemistry, 3rd Edition, 1990, ISBN 0915274531.
 - d. **An unsuspected disease or complication?**
Useful references:
Effects of Disease on Clinical Laboratory Tests, Friedman and Young, AACC Press, 2nd revised edition, 1989, ASIN 0915274523
Interpretation of Diagnostic Tests, Jacques B. Wallach, Lippincott Williams & Wilkins Publishers, 7th edition, 2000, ISBN 0781716594.
If the unexpected result cannot be explained by any of these considerations, go to step 4.

4. **If a laboratory error is suspected after analysis of the reported data**, please forward and order request marked: "Confirmation Request for (test) of (date and time). The request must include the physician's name and pager number.

Upon receipt of a confirmation request, the laboratory will:

- a. Check original data to detect transcription errors;
 - b. Repeat study on original sample (if available) to detect error in original determination;
 - c. Repeat determination on a new sample on the patient to detect possible error in patient or specimen identification;
 - d. If steps (b) and (c) confirm the original results, the clinical findings and drug history are reviewed for possible unsuspected disease or complications and for possible drug interferences;
 - e. The results of the confirmation are reported to the physician by the Clinical Pathology Resident (501-688-2820). (No charge is made to the patient for a Confirmation Request).
5. **Erroneous laboratory results** (whether clerical or analytical errors) which have been entered into the patient's computer record will be replaced with the corrected results. A numeric result will appear on the patient report with the notation "Previously Reported As" followed by the old result. Text results that are replaced will display the notation "Corrected Report" with the new result underneath. "Previously Reported As" will be followed by the old text result.



HEPCIDIN: The link to understanding iron regulation

Find out about the mechanisms of action of hepcidin, clinical diseases resulting from aberrations in hepcidin, and its role as a putative inflammatory marker.

By Judith Robens, MD

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or many years, the exact mechanism of iron regulation has not been well

understood. Hepcidin, a peptide produced in the liver, recently has been discovered to have a central role in iron homeostasis. It has been shown to block intestinal iron absorption, thus lowering serum iron concentrations. Hepcidin production is increased with elevated serum iron and inflammatory cytokines, particularly interleukin-6 (IL-6). Chronic inflammatory states can lead to anemia of inflammation via increased hepcidin. Conversely, mutations disrupting hepcidin production have been shown to cause hereditary hemochromatosis, a result of uninhibited iron absorption. The purpose of this review is to discuss the mechanisms of action of hepcidin, clinical diseases resulting from aberrations in hepcidin, and its role as a putative inflammatory marker.

Iron homeostasis

The body requires iron, complexed with hemoglobin within erythrocytes, to serve as the main oxygen carrier to tissue. Iron is also essential to cytochromes present within the mitochondrial electron transport chain. Erythrocytes are constantly being formed and degraded with an average life span of approximately 120 days. Despite the large volume of erythrocyte turnover, iron is sufficiently recycled such that only one to two milligrams of dietary iron per day are needed to maintain the body's approximate two to four grams of total iron. Enterocytes lining the small intestine initially absorb iron via an iron transporter, DMT1. Iron must be absorbed in its reduced state, Fe^{2+} , to pass through the transporter. Iron found from animal sources is directly absorbed; however, iron found from plant sources must first be reduced from Fe^{3+} to Fe^{2+} by a duodenal cytochrome *b1* (DCytb1) prior to absorption (see **Figure 1**).

Once inside the enterocyte, iron is transported to the basolateral side of the cell where iron can be transported out of the cell into the blood. This transport is achieved by another transmembrane transporter termed ferroportin. Iron is then bound by the plasma

protein transferrin, which serves as the source of iron for erythropoiesis and cellular respiration, taken up into cells expressing the transferrin receptor.

Ferroportin is also expressed in macrophages that have engulfed and degraded senescent erythrocytes. Ferroportin allows the macrophages to release iron so that it may be re-utilized for ongoing erythropoiesis. Hepatocytes also express ferroportin.

Hepcidin, a peptide produced by the liver and encoded by the HAMP gene, has been found to play a central role in iron metabolism and homeostasis.¹ Hepcidin production is upregulated with increased serum iron and then blocks iron transport through the ferroportin channel in enterocytes and macrophages, thus decreasing the amount of iron released into the blood. The iron is then either trapped in the enterocytes and sloughed into the gastrointestinal tract, or it is trapped in the macrophages of the reticuloendothelial system. Mouse macrophage cell cultures incubated with hepcidin show that expression of ferroportin itself is reduced several hours after incubation.²

Alterations in hepcidin production and clinical outcomes

Distinct clinical outcomes result from either too little hepcidin or too much hepcidin. Anemia of inflammation is a disorder that develops following prolonged periods of inflammation and is broadly characterized by low serum iron, low to normal transferrin, and high ferritin. There are numerous examples of clinical conditions that lead to a chronic inflammatory state, including rheumatologic autoimmune diseases, malignancies, and infections. The common feature in these diseases is production of inflammatory cytokines, such as interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), and bone morphogenic protein (BMP). Elevated levels of cytokines IL-6 have been shown to increase hepcidin levels even hours after administration.³ BMP has also been shown to raise hepcidin production in mice, independent of IL-6 function.⁴ Chronic elevation of these inflammatory cytokines, particularly IL-6, has consistently been shown to raise hepcidin levels. This subsequently acts on the ferroportin iron transporter, and inhibits iron release into the bloodstream from enterocytes and macrophages. One possible rationale for this biologic mechanism is to sequester iron and reduce iron availability for microbial metabolism. It has also been shown that high levels of hepcidin have antimicrobial activity,⁵ and it is thought that cytokine-mediated hepcidin release during infection is part of the body's defense against microbes. Ultimately, anemia of inflammation results and is generally not responsive to oral iron intake, as intestinal absorption is blocked via hepcidin.

Traditionally, medical therapies aimed at treating anemia of inflammation include high doses of erythropoietin and iron intake. The bone marrow appears to be less responsive to erythropoietin stimulated red cell production, so higher doses of erythropoietin are generally needed to overcome this. Also, as the mechanism of reduced serum iron is related to hepcidin-mediated blockade of intestinal iron absorption, parenteral iron

administration may be required to improve serum iron levels if high doses of oral iron are ineffective. Alternatively, new methods at cytokine blockade may be beneficial, as in TNF-alpha blockade currently in use for rheumatoid arthritis. Newer therapies aimed at blocking hepcidin-mediated anemia are currently being evaluated in animal models. Mice with anemia of inflammation treated with anti-hepcidin antibodies showed improvement of anemia.⁶ In cases of hepcidin-producing liver tumors causing anemia, surgical tumor resection was followed by spontaneous normalization of anemia.⁷

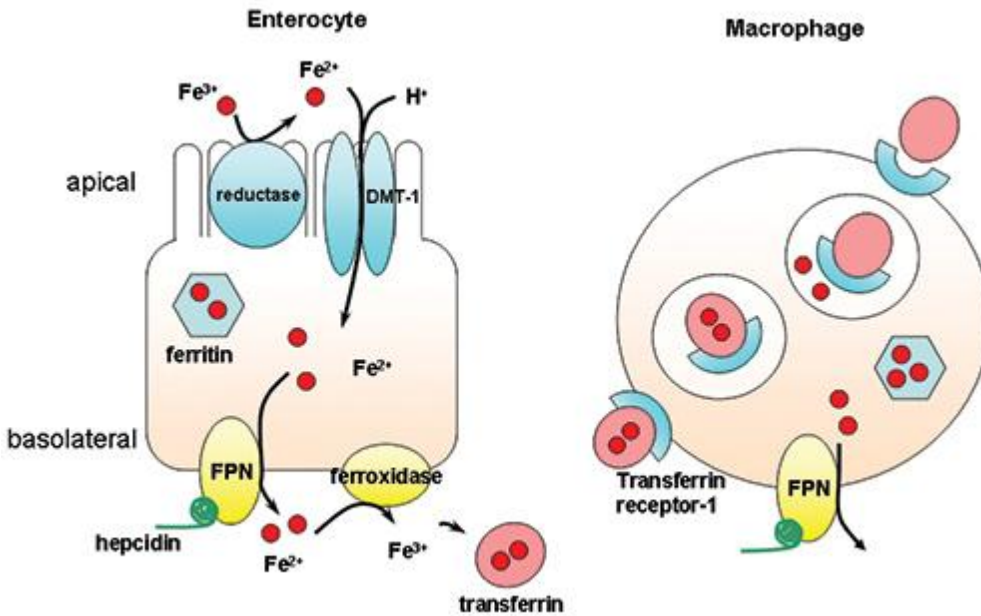


Figure 1. Iron homeostasis and regulation in an enterocyte and macrophage.

Elemental iron absorption occurs in intestinal epithelial cells (enterocytes) in the duodenum. Epithelial cells mature up the villus axis about 7 days only to be sloughed off at the tip. Iron is then transported into the blood through ferroportin (FPN) or lost with the sloughed enterocyte. Macrophages and cells expressing transferrin receptor-1 (TfR-1) internalize the transferrin-iron complex. Iron is released within the vesicles and will either be stored within the cell complexed with ferritin, or will be released into the blood through the ferroportin channel. Hepcidin prevents ferroportin mediated release of iron.

Conversely, when gene mutations occur in the hemochromatosis iron protein (HFE), hemojuvelin, or hepcidin; hepcidin production is knocked out or reduced. Hepcidin then is not available to block iron transport through the ferroportin channel into the bloodstream. As the body does not have an effective mechanism to dispose of excess iron, the iron is deposited throughout the body. The iron-overload syndrome known as hemochromatosis then develops, leading to complications such as liver failure with

cirrhosis, constrictive cardiomyopathy, gray discoloration of the skin, and diabetes from pancreatic dysfunction.

Mutations directly involving the hepcidin HAMP gene account for a small percentage of cases,⁸ indicating that mutations in hepcidin regulatory genes are mainly implicated. These affect the expression of hepcidin, leading to clinical hemochromatosis. Indeed, HFE is responsible for transcriptional regulation of hepcidin.⁹ Patients with HFE-hemochromatosis (most commonly due to C282Y mutations) have decreased hepcidin/ferritin ratios compared to normal patients, indicative of inappropriately low hepcidin in the face of elevated iron stores. Iron challenge in these patients increases urinary hepcidin in an incremental fashion according to their HFE homozygous, heterozygous, or wild-type gene status; this supports hepcidin production regulation by iron levels.¹⁰ Several studies have suggested that hepcidin production is decreased in hereditary hemochromatosis with HFE mutations due to defective BMP or BMP blockade.^{11,12}

Mutations in the ferroportin gene render the ferroportin iron transporter unresponsive to hepcidin and may also lead to hemochromatosis. Although the exact function of transferrin binding protein transferrin receptor 2 (TfR2) is not fully known, homozygous mutations are also a cause of hereditary hemochromatosis.¹³ In mice with homozygous mutations in TfR2 (TfR2 $-/-$), introduction of TfR2 gene via a viral vector increased hepcidin levels and decreased transferrin levels compared to control mice, thus supporting a role in hepcidin regulation.¹⁴

While classic treatment for hemochromatosis includes therapeutic phlebotomies to rid the body of excess iron, knowledge of the role of hepcidin may aid treatment in the future. In mice with HFE $-/-$ hemochromatosis, daily administration of purified hepcidin for two months normalized serum iron levels.² While the hepcidin administration did reduce serum iron, hepatic and tissue iron stores did not decrease during the two-month treatment course, consistent with hepcidin trapping iron in hepatocytes and macrophages via ferroportin blockade. Whether hepcidin can be eventually used as a treatment for hemochromatosis remains to be determined.

Hepcidin as an inflammatory marker

As previously mentioned, hepcidin levels have been shown to be increased as a result of inflammation and may have antimicrobial activity. Additionally, hepcidin itself may serve as an inflammatory marker. Patients with renal transplants frequently develop coronary artery disease (CAD), a condition that is considered an inflammatory state. Patients with renal transplants were found to have higher levels of hepcidin in those with CAD than those without, and the higher levels of hepcidin also correlated with higher levels of known inflammatory markers.¹⁵ Other patients with renal failure receiving hemodialysis had higher mean hepcidin levels compared to controls. Hepcidin levels

were reduced following hemodialysis¹⁶ but returned to baseline levels within one hour following hemodialysis.¹⁷ Elevated hepcidin levels correlated with elevated C-reactive protein and IL-6, yet hepcidin was also elevated in a subset of hemodialysis patients without microinflammation and correlated with ferritin.¹⁷ Another study of patient's with Hodgkin's lymphoma found a significant correlation between elevated IL-6 levels with hepcidin but not with other cytokines IL-10 and thymus and activation-regulated cytokine (TARC).¹⁸ Hepcidin was also significantly correlated with increased fibrinogen, another known acute-phase reactant.

Analytical issues

As the clinical relevance of hepcidin has become apparent, refinement and standardization of hepcidin analysis is essential. There are multiple methods currently employed by different laboratories to measure hepcidin levels, including weak cation exchange chromatography, time-of-flight mass spectrometry (TOFMS), and immunochemical methods.¹⁸⁻²⁰ A recent study by Kroot, et al, looked at the correlation of hepcidin levels of 15 samples from urine and plasma sent to different laboratories in six different countries.²⁰ Samples were taken from both healthy subjects and patient subjects, and covered a wide range of hepcidin concentrations. Different methods used in the analysis included both mass spectrometry and immunochemical methods. Samples were tested in replicates and showed generally fair precision within each specific method, yet concordance with lab values obtained from different laboratories showed considerable variation. For example, one sample's values ranged from 11.6 nmol to 1646.9 nmol hepcidin/nmol creatinine. This problem exemplifies that hepcidin measurement has not been standardized and may be due to a number of causes, including assay differences and different calibration solutions. Therefore, comparing hepcidin levels from different laboratories must currently be analyzed within the context of the laboratories' reference ranges.

Immunoassays have shown some difficulty in anti-hepcidin reagents as the hepcidin molecule is rather small (25 amino acids). Additionally, antibody development is challenged by hepcidin conservation among animal species. Efforts at improving reproducibility by spiking a urine or serum sample with synthetic hepcidin-24 are currently being studied for TOFMS.¹⁹ This method allows for hepcidin-24 to serve as an internal control to better account for hepcidin-25 variability following sample processing.

Conclusion

Hepcidin is a small protein produced in the liver that has emerged as a central regulator of iron homeostasis. It prevents iron release into the bloodstream by blocking the iron transporter ferroportin, present on the basolateral surface of enterocytes as well as macrophages and hepatocytes. Iron is then either shed with the enterocytes in the gastrointestinal tract or trapped within macrophages and hepatocytes.

Hepcidin production is upregulated in response to iron deficiency and inflammatory cytokines, particularly IL-6. Diseases with chronic elevation of inflammatory cytokines (e.g., rheumatologic disorders, malignancies, inflammatory bowel disease, and others) lead to hepcidin upregulation and, consequently, anemia of inflammation. Conversely, mutations in hepcidin regulatory genes HFE and hemojuvelin in which hepcidin production is inhibited or knocked out, lead to the iron-overload condition: hereditary hemochromatosis. Efforts at mitigating the over-production or under-production of hepcidin via IL-6 blockade or hepcidin infusion may prove useful in the future in the treatment of anemia of inflammation and hereditary hemochromatosis, respectively.

Last, laboratory measurement of hepcidin is currently not standardized. Although values within method may be reasonably reproducible, hepcidin measurements between different institutions are not currently comparable. Studies to improve standardization and improve methods for hepcidin measurements are needed.

Judith Robens, MD, is currently a member of the anatomic and clinical pathology residency training program at Beth Israel Deaconess Medical Center and Harvard Medical School. She completed her doctorate of medicine at the University of Miami Miller School of Medicine in 2007.

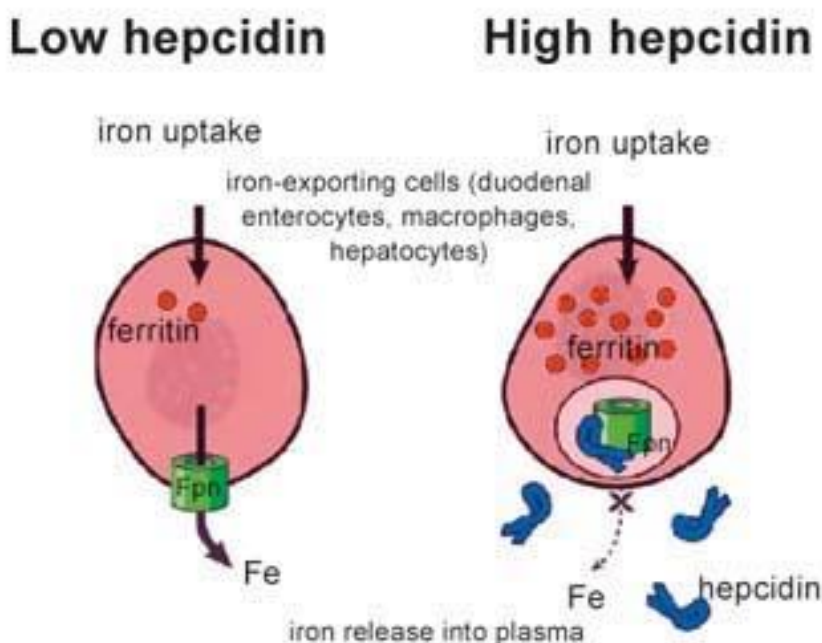
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Figure 1 - Mechanism of hepcidin-mediated cellular iron regulation



Fpn = ferroportina (adaptado de Ganz ³).

Reshaping healthcare at the clinical laboratory level

By Nilam Patel, MT(ASCP)SH and Barbara Connell, MS, MT(ASCP)SH

Proposed new rules for accountable care organizations (ACOs) released by the U.S. Department of Health and Human Services (HHS) March 31, 2011,¹ are designed to help doctors, hospitals, and other healthcare providers better coordinate care for Medicare patients across care settings — including doctors' offices, hospitals, and long-term care facilities. The Medicare Shared Savings Program will reward ACOs that lower healthcare costs while meeting performance standards on quality of care and putting patients first.

As ACOs continue to be noted in national dialogue as a model that may reshape healthcare by helping to improve quality of care, increase efficiency, and reduce overall healthcare spending, we might ask what clinical laboratory departments can do to create their own “accountable care” laboratory models, which, in turn, would contribute to the efficiency and productivity of their hospitals overall. To answer this question, clinical laboratory professionals will want to consider reshaping their laboratories with clinical, operational, and financial foci.

Clinical excellence

The spectrum of clinical excellence begins with the core technology and clinical utility of a laboratory's instrumentation and ends with diagnosis, treatment, and improved quality of life for the patient. As such, reliable best-in-class automated hematology systems with advanced, clinically relevant parameters that can potentially impact treatment guidelines, care pathways, patient flow, and return on investment are essential. Clinical laboratories will want to ensure that these advanced, clinical parameters can be automatically measured in the course of routine hematology testing and require minimal additional capital, labor, or reagents.

Refinement of already-embedded technologies has presented hematology laboratories with three new clinical parameters: reticulocyte hemoglobin (RET-He), immature platelet fraction (IPF), and immature granulocytes (IG). All three are fully reportable and all have

one thing in common: the ability to analyze cell precursors to provide physicians with more information to help drive treatment decisions and a positive patient outcome. These three parameters meet key test criteria: clinical relevance, reliability and speed, and affordability.

Reticulocyte hemoglobin (RET-He)

Whether automated or performed manually, the reticulocyte count has been a mainstay for studying anemias and anticipating recovery of the red-cell population. A closer look at the reticulocyte population, however, can provide more information than the reticulocyte count alone since red-cell production does not necessarily mean the iron carrying capacity of the red-cell population is certain. Nor do iron-store estimates provide an assessment of the availability of iron for introduction into developing erythrocytes. Significant, rapid increases in red-cell production can overwhelm available iron stores, resulting in a hypochromic anemia due to functional iron deficiency².

RET-He is a measure of the hemoglobin content of reticulocytes and is a direct assessment of the incorporation of iron into erythrocyte hemoglobin, reflecting recent functional availability of iron. A study performed at DaVita and published in the American Journal of Kidney Diseases³ demonstrated the clinical application of RET-He, by physicians, as an indicator for anemia treatment decisions in end-stage renal disease patients on hemodialysis. RET-He is now an established parameter in the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines for assessing the initial iron status of hemodialysis patients with chronic kidney disease. It is also used to assess IV iron replacement in these patients versus measuring storage or transport proteins, serum iron, or using algorithms which incorporate indirect assumptions. RET-He is now a routinely reported parameter.

Immature platelet fraction (IPF)

Platelet counts have been a challenging parameter for hematology instruments, particularly for low platelet counts. Recently, clinicians have been looking at the IPF as an indicator of imminent platelet recovery and as an indirect means to better evaluate the need for platelet transfusion.

In 1992, Ault, et al,⁴ publishing in the American Journal of Clinical Pathology, coined the term "reticulated platelets" to describe platelets with elevated nucleic-acid content. This platelet population also is characterized as having larger, more physiologically active platelets and is analogous to the red-cell immature reticulocyte fraction. This immature population is now referred to as the immature platelet fraction (IPF) and can be measured automatically through routine hematology analyzers. IPF is now accepted as an indicator of the rate of thrombopoiesis and platelet-count recovery. It is also able to help determine

the etiology of thrombocytopenia by differentiating increased platelet destruction versus increased platelet consumption. The IPF, along with other clinical signs and symptoms, may also have the potential to be used by the physician to limit platelet transfusions in the recovering patient.

Immature granulocytes (IG)

Manual counts in the hematology laboratory are becoming a thing of the past now that the abnormal white-cell differential is automated. Instruments are able to identify and provide an absolute count and percent of the IG population comprised of metamyelocytes, myelocytes, and promyelocytes. Analysis is based on their increased fluorescence emission due to higher levels of DNA and RNA versus mature neutrophils. The measurement is made in seconds and is reported automatically with the other CBC results. This ability allows for enumeration of a large number of neutrophils at various levels of maturity — certainly beyond that possible with a 100-cell manual differential — and is critical in neutropenic samples. Furthermore, this comprehensive reporting may help improve laboratory productivity by eliminating manual reviews on systems that rely on "flags" to indicate the need for a manual examination for immature granulocytes, some of which will be false-positives.

In a study by Briggs published in *Laboratory Hematology* of 210 patients known to have infection or inflammation, the IG counts were often positive (>2%) when other known markers of infection (CRP, ESR, CD64, and IL6) were negative.⁵ Now that the lab is able to report the IG population with a high degree of accuracy and precision, the clinical utility of the white-blood-cell (WBC) count and differential can significantly increase, giving physicians additional diagnostic parameters to consider when assessing inflammation and acute infection for timely intervention with appropriate therapeutics. It is evident that the clinical utility of automated hematology parameters is increasing and being efficiently generated.

Operational efficiency

From an operational perspective, efficiencies within high-volume testing departments such as hematology are highly measurable. Specific measurements that are taken within this department include turnaround times, staffing utilization, operational costs, workflow efficiency, and productivity. Quality is also tightly controlled and it is enhanced with the application of LEAN and Six-Sigma principles to further improve processes by reducing errors. Additionally, hematology laboratories depend on automation and standardization to maximize efficiencies.

With these kinds of metrics already in place, we might ask, “How much more operationally efficient can a hematology laboratory be?” An integrated hospital network (IHN) provides a good example. From New York to the state of Washington, IHNs are reshaping their testing environments to address forecasted medical technologist shortages. The American Society for Clinical Pathology’s 2011 Vacancy Survey of U.S. Clinical Laboratories⁶ surveyed 625 facilities. Of the eight laboratory disciplines studied, hematology ranked fourth with nearly 7% vacancy rates reported. Of those working in hematology laboratories, 14.4% are expected to retire within the next five years.

More efficient instrument systems that generate high quality, reproducible results from all laboratory sites across a system will help hematology laboratories shift personnel between laboratory venues without the need to cross train on different types of analyzers; withstand future medical technologists’ shortages by handling increased workloads without the addition of personnel; and keep up with workload demands and testing complexity that may accompany our country’s aging population.

Costs and labor pressures will continue to weigh heavily on IHN laboratories. In response, labs will seek automation to minimize “test-tube touch-points” and improve standardization within the laboratory and across the enterprise. These challenges can best be met through a laboratory strategy that focuses on sample- and data-process control.

Standardization is achieved by using instrumentation with similar technologies, while offering different levels of sample throughput. Labs, especially those in large integrated health networks, are looking for standardization and scalability across the enterprise. IHNs require instruments that produce the same quality results (using the same technologies, the same reagents, and the same software-management system) and have the ability to scale up and down, depending on the test volume at the individual lab location. Applied rules must be the same 24/7 and across all instrumentation. Samples need to be handled the same way, regardless of shift or day of the week.

IHN medical technologists review a variety of data to validate results: instrument flags, demographic data, and comparison with previous results without subjective results variations. The time-consuming process of validation of manual, paper-based result comparison and unnecessary rerun testing needs to be eliminated. An instrument solution should help address these labor intensive, error-prone processes and have the ability also to manage the tube from pre- through post-analytical phase.

Hematology laboratories can achieve dramatic results and raise process standardization by applying rules-based decision-making software within their testing environment. The rules engine should be robust and comprehensive and should be able to handle complex decisions such as evaluating whether a smear needs to be prepared based on the

“presence” of an instrument flag and/or a combination of demographic indicators and whether a slide was “not” made previously within a laboratory’s defined time frame.

Multivariable rules can be used to autovalidate 70% to 80% of hematology results, releasing them to the chart without operator intervention allowing technologists to focus on the remaining 20% to 30% of variant results that require additional decision-making. Labs can achieve dramatic results when combining many variables to build their rules, which can be very specific and may be adapted as the laboratory changes.

At New York Hospital Queens (NYHQ), a part of the New York-Presbyterian Health Care System, more than 2.3 million tests are performed yearly. The hematology department processes over 1,000 samples per day including 1,600 hemoglobin A1cs (HbA1c) per month on a fully automated lavender-top track system. The “line” consists of two advanced technology hematology analyzers, a slide-maker stainer, a hemoglobin A1c instrument, a tube sorter/archiver, and decision-logic software. The laboratory also has an automated digital-imaging system to help improve efficiencies and achieve standardization with its smear-review process. This comprehensive automation solution achieves a higher level of efficiency, process standardization, and staff utilization analyzing >90% of tests from lavender-top tubes and 100% of pre- and post-analytical sample handling. The laboratory is now autoverifying 85% to 90% of both CBCs and HbA1c results. The entire line is managed by only two technologists during peak workloads. According to Alfonso Ziccardi, MT(ASCP), assistant laboratory operations manager at NYHQ, true laboratory standardization is achieved by applying the same decision-making rules across the system.

In conjunction with standardization of instruments, IHNs need to standardize review and management of quality-control (QC) practices and results. Middleware-managed QC software, using system-wide rules that consistently evaluate QC across all labs, shifts, and technologists, meets these needs. Standardized QC qualification enables the IHN to measure instrument precision across the same lot numbers and across all sites by instrument type. This information can provide the ability to quickly identify QC trends (proactively versus retrospectively) to minimize risk to patient reporting. An enterprise view of QC viewed both graphically and statistically in real time, provides one more tool to help improve medical outcomes.

On the West Coast, PeaceHealth Laboratories recently standardized hematology testing across all nine of their laboratory sites. PeaceHealth installed an automated hematology track system in its high-volume core lab and three same-technology analyzers from the same vendor to accommodate workload variations at its other sites. PeaceHealth, a not-for-profit IHN which services Alaska, Washington, and Oregon, consistently receives

national recognition for innovations in patient-centered care, patient safety, healthcare technology, and cost efficiency.

One PeaceHealth hematology laboratory, Longview, under the direction of Jerry Pittman, MT(ASCP), has managed a 40% increase in volume — and the implementation of new clinical parameters — with no additional staff and no prolongation of turnaround times. Pittman believes that recent advances in automated differential analyzers that allow for quantitative IG reporting has resulted in the biggest net efficiency change in how his hematology laboratory functions. By using all of the information the instrument provides, not just quantitative cell counts, he has cut manual smear reviews virtually in half. With decision-making, rules-based efficiency, and a significant amount of validation data on file, this hematology laboratory is now reporting up to 4% IGs without operator review due to the confidence they have in its analyzer.

By standardizing instrument platforms, information integration, and advanced technologies, integrated health networks can achieve unexpected levels of optimization for laboratory operations that literally transform their productivity.

Reimbursement and survival

In October 2008, the Centers for Medicare and Medicaid Services introduced significant restructuring of the diagnostic-related groups (DRGs) to medical-severity diagnosis-related groups (MS-DRGs) used in the inpatient prospective payment system (IPPS). New present-on-admission (POA) rules require evidence-based patient diagnosis on admission. This will impact how hospitals code patients upon admission and, consequently, the way in which hospitals are reimbursed. This, along with an aging American population, increasingly complex cases and diminished human and financial resources, make accurate patient diagnosis and instrument capability critical elements to adequate reimbursement and hospital survival.

Anticipating this need at NYHQ, the hematology laboratory is now conducting a study of the IG parameter and its ability to correlate with a positive blood culture, so that if a person arrives in the emergency department with an infection, the lab will have another tool to use to identify on admission that the infection was pre-existing and does not constitute a hospital-acquired infection, or HAI, which has reimbursement issues.

The crucial question

As the nation's leaders continue their endeavors to reshape healthcare via new models of efficiency such as ACOs, clinical-laboratory instrument manufacturers must ask the question: "How is the clinical laboratory going to evolve over the years to come, and what can we do to shape and support that development?" Will new cellular studies that

are able to assess red cell, white cell, and platelet precursors, and provide quantitative assessments usher in an age of “predictive hematology” that could significantly impact therapeutic choices and lessen interventional studies, contributing to patient safety? Manufacturers will need to make significant technology changes that impact clinical outcomes and costs. As such, clinical laboratories will want to make purchasing decisions based on a total “solution” strategy rather than on technology and throughput alone.

More than ever before, healthcare providers must balance the need to provide quality patient care with the need to deliver that care as efficiently and cost effectively as possible. These needs are not going to change, so it is the instrument manufacturers’ responsibility to provide products and service that help meet these needs today and in the long-term. Clinical labs, in turn, can create accountable-care laboratory models that have the potential to reshape healthcare at the clinical-laboratory level. By thoroughly considering reliable automated hematology systems with advanced, clinically relevant parameters, clinical laboratories can potentially impact treatment guidelines, care pathways, patient flow, and return on investment.

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The anarchy of antibiotic resistance: mechanisms of bacterial resistance

By Cynthia B. Schofield, MPH, MT(CAMT)

From *Staphylococcus aureus* resistance to penicillin in the 1940s and

methicillin resistance in the 1960s, the focus of antibiotic resistance has been on Gram-positive bacteria. Glaring reports of methicillin-resistant *S aureus* (MRSA) infection aroused public awareness and brought research priorities to the forefront in the 1970s and 1980s. Yet another exigency arrived in the 1990s with the increasing appearance of the extended-spectrum β -lactamases (ESBLs), enzymes carried by Gram-negative bacteria belonging to the Enterobacteriaceae family. These multidrug-resistant (MDR) bacteria have created devastating disease, both hospital-acquired (HA) and community-acquired (CA), and are of greatest concern to infectious-disease specialists and public-health officials, worldwide.

Gram-negative bacteria have the potential not only to develop chromosomal resistance but also to quickly spread resistance via genetic determinants carried on plasmids or transposons (i.e., pieces of DNA that jump from one strain or species to another). Rapid spread through human travel and migration, combined with the looming dilemma of “no new drugs,” these resistant elements have created a global treatment crisis — greater than from Gram-positive bacteria.

Reported first in India in the 1990s, the ESBL gene *bla*_{CTX-M-15} jumped from chromosome to plasmid through a process that expedited its worldwide spread. The CTX-M15 enzyme, which continues to be the dominant ESBL, is responsible for acquired resistance to third-generation cephalosporins in the Enterobacteriaceae. In India, the presence of 70% to 90% of resistant ESBLs made treatment with carbapenem antibiotics mandatory. Most frightening, the once successful ESBL therapy has resulted in selection for resistance to the carbapenems, leaving few alternatives in the antibiotic armamentarium.¹

A large study in 2010 identified more than 150 isolates of a new metallo- β -lactamase (MBL), named NDM-1 from India, the U.K., and Pakistan. The characteristic gene, *bla*_{NDM-1}, is carried on plasmids and easily transferred among many bacteria, in a manner similar to the spread of the CTX-M15. This typical and flexible type of transmission

creates tremendous potential for the rapid spread of resistant genes throughout the environment. Carbapenem-resistant *Klebsiella pneumoniae* and plasmids that encode Verona integron-encoded MBL(VIM) in *K pneumoniae*, already found in the U.S. Greece, Israel, et al, are responsible for escalating the present healthcare crisis.¹

Arrival of NDM-1

In 2007, a zinc-dependent MBL gene, *bla*_{NDM-1}, was isolated in New Delhi, India, from the urine culture of a 59-year-old patient who had type II diabetes and a history of strokes. Although his residence was in Sweden, he often traveled to India, his home of origin. During a December 2007 trip, he was hospitalized in Punjab with a gluteal abscess. Later admitted to a New Delhi hospital (for a second surgery), he developed a decubitus ulcer (deep wound). In January 2008, he was referred to Orebro, Sweden, where he was treated parenterally with amoxicillin-clavulanic acid, metronidazole, amikacin, and gatifloxacin. At that time, a carbapenem-resistant *K pneumoniae*, characterized as an MBL *unlike any known MBL*, was isolated from a urine culture.

The new “subgroup” was designated NDM-1 or *K pneumoniae* 05-506, *bla*_{NDM-1}, which was carried on a large plasmid (180 kb) and easily transferred to other species. The patient had not experienced symptoms of urinary-tract infection, or UTI, but the isolate exhibited resistance to all antibiotics on the Gram-negative formulary — except the fluoroquinolones and colistin. More important, the isolate was carbapenem resistant and positive by the MBL E-test (AB bioMérieux), determined by clinical laboratory testing. (NDM-1 binds tightly to most cephalosporins and penicillins but less to the carbapenems). An ESBL-producing *Escherichia coli* and *Acinetobacter* sp., carbapenem susceptible, were isolated from his wounds, and the same *E coli* was recovered in a culture of otitis fluid.

He was discharged to a nursing home in March 2008, where a new urine culture grew an ESBL-producing *K pneumoniae*. A fecal specimen grew an NDM-1 producing *E coli* (on a 140-kb plasmid), suggesting an *in vivo* conjugation transfer from the 180-kb *K pneumoniae* 05-506 had taken place, but the 05-506 strain was not recovered from any subsequent cultures.²

NDM-1 is described as a “transmissible genetic element” rather than a single bacterium because of its potential to encode multiple resistance genes. Though its origin was in New Delhi, many isolates have been reported throughout India, Pakistan, Bangladesh, the U.K., the U.S., Israel, and Turkey, some susceptible only to colistin. Other determinants found in that first isolate included the broad spectrum β -lactamase, CMY-4, and genes inactivating erythromycin, rifampin, ciprofloxacin, and chloramphenicol. The same

element also encoded an efflux pump, a mechanism adding further resistance along with “growth promoters” that would ensure transcription of the genes carried by the element.²

Because of the outbreaks of carbapenem-resistant ESBL and *K pneumoniae*, the rapid spread and increased resistance of NDM-1 raised a critical question regarding the potential for controlling CA UTI, henceforth. Without new antibiotics and upgraded public-health measures, the fear of another “superbug” epidemic became decidedly real.³

Detection and diagnosis: MBLs and KPC

Although identification of Enterobacteriaceae isolates is reliable by automated or manual (conventional) methods, antibiotic-susceptibility testing in certain resistant Gram-negative bacteria requires special processing. Presence of an ESBL or AMP-C producing isolate (i.e., resistance to first-, second-, third-, or even fourth-generation cephalosporins, plus aztreonam, ertapenem, and imipenem) may lead to misinterpreted results when tested with automated systems. Recommended instead are double-disk and combination-disk methods of detection, performed with ATCC quality-control organisms.⁴ [ATCC is a private, non-profit biological resource center and research organization in Manassas, VA. ATCC’s microorganism collection includes more than 18,000 strains of bacteria. Go to [About ATCC](#) for more information.]

Bush-Jacoby-Medeiros (Integron) system	Ambler system	Enzyme activity — Examples
Group 1 cephalosporinases	C	Chromosomal AmpC resistance (usual) to β -lactams, except carbapenems; not inhibited by clavulanic acid (e.g., <i>Enterobacteriaceae</i> , except <i>Salmonella</i> and <i>Klebsiella</i>)
Group 2 penicillinases	D	Staphylococcal penicillinases; TEM-1, TEM-2, SHV-1 (broad-spectrum); TEM and SHV variants (e.g., ESBLs, 1 cephalosporinase inhibited by clavulanic acid; carbapenemases inhibited by clavulanic acid)
	A	Oxacillin-hydrolyzing, OXA; cloxacillin; ESBL cephalosporins; weak activity for carbapenems (e.g., <i>A baumannii</i>)
Group 3 metallo- β -lactamase	B	Zinc-dependent carbapenemases; resistant to inactivation by clavulanic acid, sulbactam, tazobactam, some to aztreonam; (<i>bla</i> _{VIM-1} , <i>bla</i> _{VIM-2}) <i>Pseudomonas aeruginosa</i> ... IMP (e.g., <i>Pseudomonas putida</i> , <i>Serratia marcescens</i> , <i>A baumannii</i> , <i>K pneumoniae</i> , <i>Klebsiella oxytoca</i> , et al. NDM-1 in <i>E coli</i> , <i>K pneumoniae</i> , <i>Enterobacter cloacae</i>)

Table 1. Classification of β -lactamase enzymes

Adapted from Rice LB, Bonomo RA. Mechanisms of Resistance to Antibacterial Agents.

In:

Murray PR, Jorgensen JH,

Pfaller MA, et al, eds. *Manual of Clinical Microbiology*, 9th ed. Vol. 1. Washington DC: American Society for Microbiology Press; 2007:1114-1130. 1 ESBLs — extended-spectrum- β -lactamases.

Distinguishing the carbapenemase enzymes (Class A) produced by *K pneumoniae* from (Class B) MBL enzymes can be problematic. Thus, preliminary phenotypic screening by double-disk synergy and confirmation by PCR [polymerase chain reaction] are recommended (by the Centers for Disease Control and Prevention [CDC]) for detection. The double-disk method is performed with meropenem or imipenem disks (disk diffusion on Mueller-Hinton agar) alone and together with phenylboronic acid (PBA), EDTA, or PBA and EDTA together. Zone sizes augmented by ≥ 5 mm determine a positive result for the combined-disc test. MBL bacteria are inhibited by EDTA. The test requires stringent preparation with appropriate quality controls, followed by knowledgeable interpretation of the results.⁴ Isolates of NDM-1 may be distinguished from *K pneumoniae* by PCR and collected for surveillance and epidemiologic purposes at CDC.⁴

Ambler class	Enzyme	Function	Known organisms
A	KPC ¹	Hydrolyzes all β -lactam antibiotics; inhibited by clavulanate	<i>K pneumoniae</i> , Enterobacteriaceae
B	MBLs ² (NDM, IMP, VIM, GIM, SPM)	Hydrolyze all β -lactams except aztreonam; may be inhibited by clavulanate; require zinc for enzymatic activity; inhibited by EDTA	<i>P aeruginosa</i> , <i>Acinetobacter</i> spp, Enterobacteriaceae
D	OXA	Oxacillin hydrolyzing; less able to hydrolyze carbapenems	<i>P aeruginosa</i> , <i>A baumannii</i> , Enterobacteriaceae

Table 2. Classification of carbapenemases (metallo- β -Lactamases — MBLs)

Adapted from Rice LB, Bonomo RA. Mechanisms of Resistance to Antibacterial Agents. In: Murray PR, Jorgensen JH, Tenover FC, Tenover FC, eds. *Manual of Clinical Microbiology*, 9th ed. Vol. 1 Washington DC: American Society for Microbiology Press; 2007:1114-1130.

¹KPC—*Klebsiella pneumoniae* carbapenem-resistant

²MBL—Metallo- β -lactamases (MBLs) (e.g., NDM-1, IMP, VIM, and so forth)

In the case presented here, identification of KPC 05-506 was accomplished with the Phoenix and BD automated systems. Susceptibility testing was performed with E-test strips (AB bioMérieux). The MBL test (double-disc synergy with imipenem-EDTA) and the modified Hodge test (clover-leaf formation) were used to screen for carbapenemase production. The E-test determined susceptibility MICs [minimal inhibitory concentration] added to a spectrophotometry analysis to confirm carbapenemase activity.²

Mechanism	Activity	Antibiotics affected	Examples
Enzyme inactivation or degradation	Splitting of amide bond in β -lactam antibiotics.	β -lactams, chloramphenicol	β -lactamase (TEM-1) GNRs; <i>Haemophilus influenzae</i> , <i>Neisseria gonorrhoeae</i> ; ESBLs ² (TEM-3, SHV-1 & 2, OXA-1, K1(OXY-1) CTX-M) <i>E coli</i> , <i>K pneumoniae</i>
Decreased cell membrane permeability	Porins (protein channels) in outer layer of GNRs allow hydrophilic and small molecules in; block larger molecules. Decreased uptake.	Imipenem — small	PSE-1, <i>Pseudomonas</i> spp.
		Carbenicillin — large	CARB-4 <i>P aeruginosa</i> , <i>S marcescens</i>
		Aminoglycosides	<i>P aeruginosa</i>
Efflux	Membrane transport system acts to pump antibiotics out of cell	Tetracycline from GNRs; macrolides from GPC ³	<i>E coli</i> , <i>Shigella</i> spp. <i>S pneumoniae</i> and <i>S aureus</i>
Altered target sites (caused by point mutation)	Alteration of a ribosome disrupts inhibition of protein synthesis and cell growth.	Tetracycline Aminoglycosides	<i>S aureus</i> , <i>Bacteroides fragilis</i> , <i>Clostridium perfringens</i> , <i>Helicobacter pylori</i> <i>Enterococci</i>
Altered target enzymes	Target proteins in peptidoglycan layer (PBPs ⁴); decreased affinity for antibiotic; can be lost, replaced, or induced by exposure (e.g., growth at lower temperature).	β -lactams Quinolones	<i>S pneumoniae</i> , <i>mecA</i> in <i>S aureus</i> (MRSA) <i>Enterococcus faecium</i> , <i>N gonorrhoeae</i> , <i>Neisseria meningitidis</i> , <i>H influenzae</i> ; DNA gyrase, <i>gyrA</i> in GNRs, <i>S aureus</i>
Protection of target site	Interference with binding to ribosome.	Tetracycline resistance	<i>tetM</i> gene protects ribosome
		Plasmid-mediated quinolone resistance	protects from DNA gyrase binding
Overproduction of target site	Chromosomal genes produce excess enzyme; compete with para-aminobenzoic acid.	Sulfonamides	DHPS ⁵ <i>folP</i>
		Trimethoprim	DHFR ⁶ <i>folA</i>

Table 3. Mechanisms of resistance

Adapted from Opal SM, Medeiros AA. Molecular mechanisms of antibiotic resistance in bacteria. In: Mandell GL, Bennett JE, Dolin R, eds. Principles and Practice of Infectious Diseases, 6th ed. Philadelphia, PA; Elsevier Churchill, Livingstone Inc. 2005;(1):253-270.

¹GNRs — Gram-negative rods

²ESBLs — extended-spectrum- β -lactamases

³GPC — Gram-positive cocci

⁴PBP — penicillin-binding protein

⁵DHPS — dihydropteroate synthesis

⁶DHFR — dihydrofolate reductase

Isolates were screened by PCR for presence of the mobile MBL genes (*bla*_{VIM}, *bla*_{IMP}, *bla*_{SPM}, *bla*_{GIM-1}, *bla*_{SIM-1}, *bla*_{AIM-1}) and beta-lactamase genes (*bla*_{CTX}, *bla*_{CMY}, and so forth). Further tests included DNA cloning with restriction endonucleases, PCR amplification and sequencing, pulse field gel electrophoresis (PFGE), Southern-blot analysis, and multilocus sequence typing, or MLST. When the analysis was complete, two types of β -lactamase resistance were apparent. One fragment carried the AmpC enzyme, determined by resistance to EDTA and sensitivity to the inhibitor cloxacillin.

The second fragment carried the MBL, which was resistant to the inhibitor but sensitive to EDTA in the double-disk synergy test.^{2,5}

Development of antibiotic resistance

Intrinsic (inherent) antibiotic resistance is a known phenomenon in the diverse world of bacteria. Exposure to antibiotic agents, however, creates extreme selective pressure, forcing organisms to exert a complex array of genetic manipulation to cope and evolve in an unfavorable environment. This pressure is responsible for what we know as *acquired* resistance. *Intrinsic* resistance to vancomycin in *E coli* and *K pneumoniae* negates its use as treatment for infection, unlike the *acquired* low-level resistance of *Streptococcus pneumoniae*, which can be overridden by increasing the dosage of antibiotic (e.g., pediatric ear infection).⁶

Acquired antibiotic resistance is a result of biochemical encoding in the affected bacterial genes. To this end, bacteria have devised three major mechanisms of resistance: *mutation of cellular genes*, *acquisition of resistance genes*, and *mutation of acquired genes*. *Mutation of cellular genes*, or **point mutation**, occurs in a nucleotide base pair to alter the target site of an antibiotic, thus affecting growth and activity in the cell. The early-discovered β -lactamase genes that encode enzymes TEM-1 and SHV-1 laid the groundwork for later-recognized mutant genes encoding the ESBLs of *E coli* and *K pneumoniae*, et al. (e.g., TEM-3, SHV-2, TEM-30). An example of this type (defined as a single-point mutation) also occurs when RNA polymerase is targeted by rifampin.⁶

Acquisition of resistance genes by rearrangement of large segments of DNA creates a potential for independent movement from the rest of the genome. Sequences of DNA are rearranged by inversion, duplication, insertion, deletion, or transposition from one area of a bacterial chromosome or extra-chromosomal **plasmid** to another. The NDM-1 case presented here demonstrates a rearrangement of this type.

Other mechanisms of survival: DNA may be taken up by chromosomes via recombination (e.g., penicillin and cephalosporin resistance in *S pneumoniae*) or during conjugative plasmid transfer (e.g., vancomycin resistance in *S aureus* transferred from *Enterococcus faecalis*). Like plasmids, **transposons** are extra-chromosomal, non-replicative DNA, lacking conjugative ability, able to transfer resistance genes among chromosomes or plasmids via a similar unique mobility.

Mutation of acquired genes involves **DNA acquired from extra-chromosomes** (foreign DNA) delivered to bacterial strains by plasmids, bacteriophages (bacteria-lysing viruses), naked sequences of DNA, or transposable elements (e.g., *E coli* production of TEM β -lactamase as first response to ampicillin; resistance in *K pneumoniae* mediated by β -lactamases derived by point mutation from the native TEM enzyme).^{6,7}

Epidemiology

The expanding scope of antibiotic resistance and potential for new bacterial mechanisms has added to the strategies of mobile genetic elements and integron cassettes (e.g., NDM-1), which carry multiple resistance genes. The easy transfer facilitated by plasmids or *rearrangement of large segments of DNA* allow rapid spread of resistance among multiple strains of bacteria inevitable.

Transmission of NDM-1: The potential for outbreaks and the spread of disease has been exacerbated by increasing Gram-negative resistance to most available antibiotics (colistin and tigecycline excepted) and, more critically, by the paucity of drugs in development. Adding fuel to the fire is India's history of easy-access to non-prescription antibiotics and the implications for selective resistance (e.g., the case of NDM-1).

In areas of Chennai and Haryana, India, NDM-1 has been widely spread throughout the environment. Concern mounted when the U.K. Department of Health issued a "National Resistance Alert 3" that NDM-1 had been introduced into the U.K. from India. Magnifying the prospect of international spread is travel to India and Pakistan, by both Europeans and Americans, for cost-effective elective (e.g., cosmetic) surgery.¹

Failure of epidemiologic measures: In the past, infection-control guidelines for HA and CA infections (HAI, CAI) have proven effective in limiting the spread of infection. Failure of one or more techniques (e.g., effective hand-washing by healthcare workers), however, greatly increases transmission among patients. The spread of clonally-related organisms that colonize nares (e.g., nostrils, nasal passages), skin, and feces (e.g., MRSA, MDR Gram-negatives) results from a laxity of monitored hand washing and a decreased vigilance of isolation precautions.

Day-care centers, nursing homes, schools, military bases, and prisons all have become hot beds of CAI. Overuse of antibiotic agents, particularly in nursing homes, has created a pool of highly resistant organisms, easily transported to the hospital and back.

Last, contaminated food products are the end result of selective-resistant bacteria in animals treated with antibiotics as "growth promoters." *Salmonella*, *Campylobacter* spp., *E coli* 0157 are typical Gram-negative bacteria with resistance tied to animal husbandry.⁶

New targets for therapy: Despite such measures as hospital antibiotic stewardship, which contributes greatly to preventing antibiotic overuse, the exploration of new approaches to the treatment and control of Gram-negative infection has been limited.

Human defense is based not only on enhanced infection-control measures but also in early tracking of resistance. Possible solutions include clinical laboratories' routine use of molecular technology; results available for computerized surveillance; pursuit of new targets to deter bacterial resistance; [and] decreased dependence on the pharmaceutical industry.⁶

A study based on inhibiting a key component in the biosynthesis of an essential bacterial enzyme used to create nicotinamide adenine dinucleotide, or NAD, is an example of a new pursuit. Only target enzymes of bacteria used in the experiment (e.g., *Franciscella tularencis*, *E coli*, and *Bacillus anthracis*) were affected, not human enzymes. Application of this technology may be years away, but the potential for creating a new class of broad spectrum agents exists.⁸

Investigation into the tolerance of “persister cells” affords an alternative approach to the problem of chronic infection, which is even less treatable than acute infection. Persister cells are phenotypic variants of the bacterium wild type, tolerant to eradication by antibiotics (there is no effect on the MIC) because of their dormancy. An example is relapsing infection caused by biofilms. Certain compounds known as “prodrugs” (e.g., isoniazid or ethionamide used as anti-tuberculosis therapy, metronidazole for anaerobic bacteria) are capable of killing dormant cells. The presumption follows that a similar approach targeting bacterial enzymes may counteract the efflux mechanism common in Gram-negative resistance.⁹

Conclusion

The mechanisms of resistance that allow bacteria to survive and persist in the presence of antibiotics are well documented. For many years, Gram-positive bacteria (e.g., MRSA; vancomycin-resistant enterococci, or VRE; and *Clostridium difficile*) held public attention as primary causes of HAI and CAI. Isolates from severe infection (e.g., pneumonia, sepsis, surgical wound, skin, and urinary tract), however, invariably are Gram-negative bacteria. Since the 1990s, the appearance of ESBL-producing members of the Enterobacteriaceae have made MDR (i.e., resistance to at least three classes of antibiotics [e.g., β -lactams, carbapenems, fluoroquinolones, aminoglycosides, and so forth]), a household term.

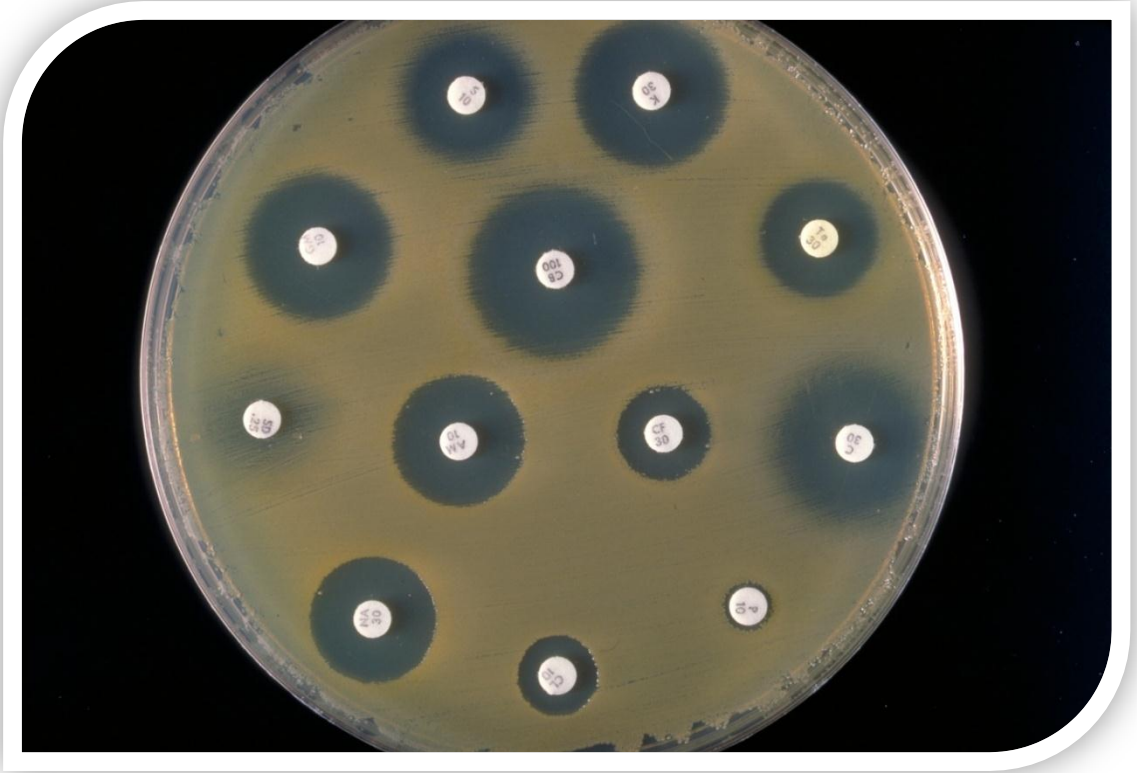
MDR bacteria range from the ESBL-producing *E coli* and *Klebsiella* spp. to include the earlier carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, as well as more recent carbapenem-resistant *K pneumoniae* and the MBL Enterobacteriaceae (NDM-1, IMP, VIM, and so forth). The endless production of enzymes by Gram-negative bacteria is solely responsible for out-of-control HAI and CAI; combined with mobile genetic elements that rapidly spread infection is the lack of

new antibiotics in development, leaving their eradication to the guidelines of infection control.¹

Poor coordination among healthcare systems, research and development, and the federal government continues to thwart the progress of targeted therapy, though reliance on pharmaceutical companies is no longer an option. Because antibiotic development is not economically feasible, searching elsewhere to solve the problem of bacterial resistance is critical to an ongoing global crisis. It bears repeating that infection-control guidelines are effective only when they are followed with scrupulous precision. Adherence to those well-established principles is documented to contain and prevent the spread of such infection as the resistant NDM-1, an organism capable of a proverbial death sentence.

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After Reading the above articles you are requested to answer the following questions and send them back to the Continuing Education committee in the PMTA , you will be rewarded 4 CE points upon successful completion of the test. Answers should be sent to: ce@palmta.org.

CE QUESTIONS

1. **Gram-negative bacteria are noteworthy for _____.**
 - a. rapidly spreading resistance
 - b. the presence of a single cell wall
 - c. gaining public attention in the 1960s
 - d. successful treatment by cephalosporin antibiotics
2. **Genetic elements that jump from species to species are _____.**
 - a. only typical of Gram-positive bacteria
 - b. rarely found in India and Pakistan
 - c. usually inhibited by antibiotic therapy
 - d. typical of ESBLs, KPC, and MBLs
3. **When NDM-1 (an MBL) is compared to CTX-M15 (an ESBL), both _____.**
 - a. are genes causing resistance to carbapenem antibiotics
 - b. are carried on plasmids to facilitate transmission
 - c. are genes causing susceptibility to fluoroquinolones
 - d. cause out-of-control urinary-tract infection
4. **Features that best describe NDM-1 include _____.**
 - a. zinc-dependence, ESBL, resistant to colistin

- b. blaNDM-1, resistant to fluoroquinolones, superbug
- c. MBL, resistant to carbapenems, carried on 180-kb plasmid
- d. CMY-4, susceptible to carbapenems, carried on 140-kb plasmid

5. The patient who often traveled to India was _____.

- a. hospitalized for a urinary-tract infection
- b. treated with carbapenem antibiotics for a wound infection
- c. treated with amoxicillin for a urinary-tract infection
- d. referred to Orebro where an MBL was isolated

6. The NDM-1 genetic element encodes multiple genes responsible for _____.

- a. inactivating erythromycin, rifampin, ciprofloxacin, and chloramphenicol
- b. urinary-tract infection in hospital and community
- c. cephalosporin, penicillin, and carbapenem susceptibility
- d. infection only in the U.K., U.S., Greece, and Turkey

7. Identification and susceptibility testing of ESBLs and MBLs _____.

- a. is reliable with conventional testing
- b. follows testing for all Enterobacteriaceae
- c. requires testing by an automated system
- d. may require double-disk synergy testing

8. Differentiating Class A (KPC enzymes) from Class B (metallo- β -lactamases — MBLs) _____.

- a. requires phenotypic double-disk screening test with PCR confirmation
- b. requires PCR screening and the E-test

- c. is accomplished with the modified Hodge test
- d. requires automated susceptibility testing and the E-test.

9. Double-disk synergy testing is performed with disk-diffusion using ____.

- a. meropenem or imipenem with PBA and/or EDTA
- b. imipenem and the modified Hodge test
- c. meropenem and PBA on Mueller-Hinton agar
- d. imipenem and EDTA on Mueller-Hinton agar

10. Identification of *K pneumoniae* 05-506 was accomplished by ____.

- a. PCR and sequencing at the U.S. Centers for Disease Control and Prevention
- b. screening for β -lactamase genes blaCTX, blaCMY
- c. multilocus sequence typing and PFGE
- d. the Phoenix and BD automated systems

11. Confirmation in the case of *K pneumoniae* 05-506 required ____.

- a. E-test susceptibility strips and disk diffusion.
- b. conventional methods and the modified Hodge test
- c. the MBL E-test and spectrophotometry for carbapenemase
- d. automated systems and PFGE

12. Susceptibility testing of 05-506 was performed by ____.

- a. conventional methods
- b. E-test susceptibility strips
- c. the Phoenix and BD automated systems

d. MBL E-test with imipenem

13. Resistance in E coli and K pneumoniae to vancomycin is ____.

- a. described as typical of intrinsic resistance
- b. similar to resistance in Streptococcus pneumonia
- c. typical of acquired bacterial resistance
- d. overcome by increasing the dosage of antibiotic

14. The major mechanisms of acquired antibiotic resistance ____.

- a. exclude transposons to transfer resistance genes
- b. do not affect target sites, e.g. point mutation
- c. were not apparent in the NDM-1 case presented
- d. include extra-chromosomal plasmids

15. Bacterial point mutations are not involved when ____.

- a. β -lactamase genes encode TEM-1 and SHV-1
- b. rearranged segments of DNA occurs (case of NDM-1)
- c. DNA is acquired from extra chromosomes
- d. the ESBLs of K pneumoniae and E coli are encoded

16. The spread of disease by resistant Gram-negative bacteria is exacerbated by ____.

- a. travel to areas of Chennai and Haryana, India
- b. the limited availability of non-prescription antibiotics
- c. the abundance of new antibiotics
- d. selective resistance to penicillin and ampicillin

17. Infection-control guidelines are effective unless _____.

- a. extended use of antibiotics is observed
- b. hand washing and isolation precautions are lax
- c. animals are treated with antibiotics for growth
- d. resistant organisms are transmitted in schools

18. Reliance on the pharmaceutical industry _____.

- a. will likely resolve the antibiotic-resistance crisis
- b. is the only defense against Gram-negative infection
- c. limits exploration of new targets for treatment
- d. will decrease transmission of CAIs in nursing homes

19. Research into a new approach for treating infection _____.

- a. requires no consideration of persister-cell targets
- b. presumes that current antibiotic therapy is useful
- c. assumes that a new class of antibiotics is effective
- d. targets bacterial enzymes to counteract resistance mechanisms

20. Since the 1990s outbreaks of HAIs and CAIs are _____.

- a. caused only by Gram-positive bacteria
- b. all attributable to carbapenem resistance
- c. often carbapenem-resistant ESBLs and MBLs
- d. due to a lack of therapeutic agents

المجهر

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