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Editorial

Evolving clinical microbiology in the Armed Forces Laboratories for rapid detection of antimicrobial resistant bugs and emerging viruses

Many emerging and unusual bacterial, viral, fungal and parasitic etiological agents of human infectious diseases are being reported in the literature which poses diagnostic and therapeutic challenges to the physicians, surgeons and clinical microbiologists.¹ Clinical microbiology got its wings with the development of Gram Stain by Sir Christian Gram in the year 1884 which helped to differentiate the Gram positive and Gram negative bacteria. This marked the beginning of the era of presumptive drug therapy. Paul Ehrlich pioneered the acid-fast stain recognition of *Mycobacteria* later on modified by Ziehl Neelsen. Neisser & Albert developed the staining techniques of *Corynebacterium diphtheriae*.² Thereafter, many enrichment liquid culture media along with solid, selective, differential, enriched culture media became available for isolation, identification and presumptive morphological classification. Subsequently, antisera were raised in animal species for the identification of *Salmonella* and *Shigella* group of microorganisms. Various biochemical tests were developed such as catalase test to differentiate *Streptococci* and *Staphylococci* and the coagulase test for the identification of *Staphylococcus aureus*.³ With the gradual evolution of microbiology services, plastic and polypropylene closures have replaced cotton plugs and in-house medium preparation has been replaced by commercially manufactured ready-to-use media. With antibiotic pressure and genetic variability of the bacterial response, an unabated tug of war between antimicrobial agents and the microorganisms began. However, the signs indicate that the evolutionary potential of the microbial world may succeed in the long run.⁴

Since 1960s, numerous innovations were made in the clinical microbiology laboratories. Molecular biology techniques hold a great promise to revolutionize the diagnosis of infectious diseases. The requirement for rapid and accurate diagnosis was considerably enhanced when therapeutic measures for a specific disease etiology came into practice. Thus, the impetus to improve the microbiological acumen was felt in the Armed Forces Medical Laboratories to identify agents of infectious diseases for early antimicrobial intervention. Clinical Microbiology over a period of years has seen a paradigm shift from manual culture, staining, in-house antimicrobial disc preparation and cumbersome laborious immunological tests to a modern era of miniaturization, plastic disposables and use

of commercial culture media. Mechanization of analytical microbiological procedures gave way to automation such as fully automated antimicrobial susceptibility testing machines, fully automated blood culture system; flow cytometric based testing, use of fluorescent microscopes, automated anaerobic culture system, line probe assay and Polymerase Chain Reaction (PCR) for molecular detection of pathogens.⁵ The viral load detection by Real-Time PCR has revolutionized the monitoring of the antiviral therapy especially in cases of Hepatitis and HIV infection. Armed Forces Laboratories now have the capability to detect H1N1 influenza. A virus, Japanese encephalitis virus, dengue virus serotypes by fully automated nucleic acid extraction systems and Isothermal loop amplification techniques and to further characterize them by automated sequencing. The advancements have been remarkable in the ability to detect emerging and unusual viral infections such as MERS and Crimean Congo Haemorrhagic viruses. Some of these laboratories have recently been upgraded with facilities to detect all multidrug-resistant bacterial strains and detection of metallo-beta lactamases producing super bugs such as NDM-1 and detection of nosocomial pathogens such as Methicillin resistant *S. aureus* (MRSA), which are frequently associated with health care associated infections.

Emergence of antibiotic resistance among the pathogens is the most serious threat to the management of infectious diseases. Penicillin was discovered serendipitously in 1928 and has been found to be lethal towards sensitive cells via specific deactivation of cell wall located penicillin binding protein (PBP) transpeptidases.⁶ Inactivated PBPs are prevented from cross linking the peptidoglycan strands that in turn leads to a weakened cell wall and subsequent death by osmosis. The implementation of penicillin as a frontline drug in the clinical treatment for *Staphylococcus aureus* (*S. aureus*) commenced in the early 1940s. By 1950s, penicillin was a well established wonder drug for treatment of infections caused due to *Staphylococcus aureus*. However this enthusiasm did not last long. Isolates that acquired a penicillin hydrolyzing enzyme (penicillinase) were favored by natural selection and led to the origin of resistant strains.⁷ By demonstrating resistance to penicillinase and PBP binding affinity, methicillin was hailed as the definitive treatment against *S. aureus* infection. But in 1961,

within 12 months of its clinical use, the first treatment failure occurred and MRSA had evolved.⁸ Resistance to Methicillin and all β -lactam antibiotics is mediated by an exogenous substitute for the intrinsic PBP, termed PBP2' or PBP2a, which is carried by a mobile genetic element termed the Staphylococcal cassette chromosome mec (SCCmec). The first MRSA strain (NCTC10442) that was isolated in the United Kingdom (UK) in 1960 harbored SCCmec type I. This MRSA clone is now known as the Archaic clone and it got disseminated worldwide in the 1960s. Since the beginning of the 1990s, several MRSA clones harboring SCCmec type IV have disseminated worldwide.

The most effective treatment presently available against multi-resistant MRSA infections is the glycopeptide vancomycin. However like penicillin and its derivatives, vancomycin resistance has also evolved. The glycopeptide vancomycin was considered to be the best alternative for the treatment of multidrug-resistant MRSA⁹ but there are increasing numbers of reports indicating the emergence of vancomycin-resistant *S. aureus* (VRSA) strains. This is due to the thickened bacterial cell wall, where many vancomycin molecules get trapped within.¹⁰ The trapped molecules clog the peptidoglycan meshwork and finally form a physical barrier towards further incoming vancomycin molecules.¹¹ A recent prospective study "In vitro vancomycin susceptibility amongst methicillin resistant *S. aureus*" by Chaudhari et al¹² at a tertiary care hospital analyzing 232 clinical MRSA isolates, published in this issue, highlights the importance of determination of vancomycin and linezolid minimum inhibitory concentrations (MIC) by agar dilution method and epsilometer (E test). All isolates were found sensitive to linezolid. Two MRSA isolates had vancomycin MIC ≥ 4 mg/ml; vancomycin MIC₅₀ and MIC₉₀ isolates was 0.5 and 0.2 mg/ml respectively by agar dilution method. There was agreement over 93.5% isolates in vancomycin susceptibility by agar dilution and E test. E test had sensitivity and positive predictive value of 1.0 (CI 0.34–1.0) and 0.5 (CI 0.17–0.83) respectively compare to agar dilution method. The study concludes that MRSA isolates continues to be susceptible to vancomycin and linezolid. E test was found equally suitable in initial screening for vancomycin susceptibility. The study has recommended ongoing surveillance of *S. aureus* with reduced vancomycin susceptibility (SA-RVS) due to geographical variation in prevalence.

This issue also highlights an original study on "Usefulness of enzyme immunoassay (EIA) for screening of anti HIV antibodies in urinary specimens" by Sahni et al.¹³ The Urine-based HIV Test was approved in 1996.¹⁴ The test uses urine samples to detect IgG antibodies to HIV-1 by ELISA. To evaluate this, 436 individuals with high-risk behavior and strong clinical suspicion of HIV infection were screened for IgG antibodies to HIV-1 in urine by enzyme immunoassay (ELISA). Urine HIV testing was performed, at the ongoing Voluntary Confidential Counseling and Testing Center (VCCTC) at a large tertiary care Microbiology lab. In all individuals, both serum and urine were tested for IgG antibodies to HIV-1. Sensitivity was found to be 89.6%; 95% CI [82.9–94.0], specificity 97.3%; 95% CI [94.6–98.8], positive predictive value 93.8%; 95% CI [87.8–97.1] and negative predictive value 95.4%; 95% CI [92.3–97.4]. The study concludes that sensitivity, specificity and over all efficacy of the urine-based screening for HIV-1 test kits were excellent as compared to the reference serum IgG test. However, a positive screening test

must be followed by a blood test to confirm the results. To ensure the quality of test results, a program consisting of quality assurance, quality control, and quality assessment is necessary.

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Air Marshal D.P. Joshi, PVSM, AVSM, PHS
Director General Armed Forces Medical Services, O/o DGAFMS,
Ministry of Defence, 'M' Block, New Delhi 110001, India
E-mail address: dhanjosshi26@gmail.com

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